WISEUSE INTERNATIONAL RESEARCH & DEVELOPMENT

18 UNITY IN DIVERSITY

THE NATURAL POWER BEHIND SUCSESS

Introduction

Wise Use International B.V. — Restoring Balance Through Nature

At *Wise Use International B.V.*, we believe that nature knows best. Our mission is to **restore vitality and balance** in soil, water, plants, animals, and even within humans—by harnessing the power of **naturally occurring microorganisms**. Whether in agriculture, composting, water purification, or animal health, our biological solutions are developed to **activate the natural processes already present in the environment**, instead of disrupting them.

Our philosophy is simple yet powerful:
Healthy ecosystems create healthy life.

We focus on **stimulating microbial life**, enabling better nutrient absorption, natural resistance against pests, stronger plant growth, and improved water and compost quality. All of our products are crafted from **100% biological, natural, and environmentally friendly ingredients**—because we believe that only the purest components can restore true balance and long-term health.

With decades of combined experience in **agriculture, poultry, and water management**, the founders of Wise Use International B.V. have developed a reliable range of products that are not only **safe and effective**, but also fully aligned with sustainable and regenerative practices.

PoCo – Proven Vitality

One of our key innovations is **PoCo**, a powerful biological formula designed to enhance plant growth, revitalize soil life, and support ecological harmony. But beyond its visible impact in the field, we wanted to understand how PoCo influences water and life energy at a deeper level.

To assess this, we turned to a **respected water engineer with over 35 years of experience**. On **May 18, 2020**, he conducted an **energetic quality assessment** of PoCo using the method of **radiesthesia**—a specialized technique used to measure the subtle energy levels in water and natural substances.

The results were striking:

PoCo demonstrated excellent vitality properties, indicating a high energetic resonance that supports life and health on a cellular and environmental level.

These findings affirm what we see in practice: PoCo doesn't just improve growth—it **energizes, restores, and revitalizes** everything it touches.

Statement from the CEO

"From a young age, I was fascinated by the hidden intelligence of nature—the way elements transform, energy flows, and life renews itself. This passion for alchemy stayed with me and evolved into a deep respect for the natural world and its unseen forces. With over 30 years of experience, I founded Wise Use International B.V. to bring that vision to life: to create products that work in harmony with nature, not against it. Today, Wise Use is the embodiment of that dream—restoring balance in soil, water, plants, and animals by activating the power that's already present in life itself."
— **Dorotheus Wisman**, CEO & Founder, Wise Use International B.V

SDG focus
♦ SDG 1, No poverty
♦ SDG 2, Zero hunger
♦ SDG 3, Good health and well being
♦ SDG 6, Clean water and sanitation
♦ SDG 9, Industry Innovation and Infrastructure
♦ SDG 11, Sustainable City's and Communities
♦ SDG 12, Responsible consumption and production
♦ SDG 13, Climate action
♦ SDG 14, Live below water
♦ SDG 15, Live on land

Investment strategy
♦ Product presentation
♦ Strategic partnerships
♦ Research with Universities and organizations

Sustainable business plan development
♦ Development of sustainable business plans for strategic partners

What we offer Wiseuse International
♦ Knowledge transfer
♦ Sustainable Business development
♦ Workshops
♦ International network
♦ ROI
♦ Innovative Products
♦ Partnership
♦ Exclusivity

Uniting business for a better world.
Our Philosophy is Uniting Business for a better world in Unity and Diversity

18 UNITY IN DIVERSITY

- Diversity leads to creativity
- Diversity is mutual understanding
- Diversity is cultural understanding
- Diversity leads to innovation
- Diversity is knowledge and know-how
- Diversity creates unity
- Unity is the way to peace

UN GLOBAL COMPACT

United Nations Global Compact 20 years

Uniting business for a better world

#UnitingBusiness

WISEUSE INTERNATIONAL BV JOURNEY

WiseUse International BV, developed the following Join Venture partnerships for retail, research and development.

Join Ventures

- https://www.wise-use.com/about-wiseuse/
- https://www.nutrocorp.nl/
- https://www.bacagro.com/
- https://www.bacagro.ae
- https://bac-shop.nl/
- https://www.bacfertilizers.com/
- https://deltahorsepower.nl/
- https://biozar-original.nl/en

WiseUse International BV, natural power of the Successful companies above.

WiseUse International BV — Pioneering Innovation Through Research, Partnership, and Purpose

WiseUse International BV the Natural power of success

WiseUse International BV is the natural power behind several global initiatives that unite innovation with real-world impact. For over three decades, we have been dedicated to the pursuit of sustainable solutions through science, technology, and strategic collaboration.

Our success

Our success is no coincidence. It is the result of a deliberate and consistent approach: long-term investment in research and development, in partnership with international universities, governments, knowledge institutions, research organizations, farmers, cooperatives, and private sector innovators. This multidisciplinary collaboration ensures that our technologies are not only developed in the lab but also tested and proven across diverse global environments.

Keep in the Research

The philosophy of **"Keep in the Research"** defines the core identity of WiseUse International BV. We believe that true innovation is a continuous process — one that demands persistence, curiosity, and a willingness to challenge the status quo. Over the past 30 years, we have committed ourselves to this vision, investing in the people, methodologies, and systems that lead to tangible, scalable outcomes.

The value of intellectual property
This book presents a curated selection of our most significant research projects, results, and methodologies. It showcases the intellectual property we have developed, the value we have created, and the human capital we continue to cultivate. More importantly, it serves as an invitation — an open door for **investors, start-ups, and strategic partners** to collaborate with WiseUse International BV.

Open for Business
Our **Open for Business** strategy provides a unique opportunity: to co-develop and co-invest in the future, leveraging our 30 years of R&D and global networks. Together, we can create sustainable business models, accelerate innovation, and drive meaningful value — both economic and environmental.

At WiseUse International BV, we are not just building solutions — we are building futures.

We invite you to explore, engage, and invest.

STATEMENT OF COMPLIANCE

REGISTRATION NO°: CU 800845

Control Union Certification BV., hereby approves the input manufacturing done by

WISE USE INTERNATIONAL B.V.
IJSSELSTEIN, THE NETHERLANDS

Project in: The Netherlands

on the basis of inspections done at the input manufacturing unit. This approval is valid for those product(s) and area(s) that are specified in the annex. The validity of this approval solely depends on the continued compliance with the Reg. (EC) 834/2007 repealed by Reg. (EU) 2018/848; Reg. (EC) 889/2007 repealed by Reg. (EU) No 1165/2021 and is subject to surveillance inspections.

Valid until: 09th February 2026

This approval is in force until further notice, provided that the above-mentioned licensee continues meeting the conditions as laid down in the licensee contract with CU. Based on the annual inspections that CU performs, this approval is updated and kept in force.

CONTROLUNION

Date of approval
14/02/2025

Declared by
on behalf of the Managing Director

Ranjitha KS
Control Union Certification BV

Annex to Statement of Compliance

APPROVAL NO°: CU 800845 EC-INP-01-2025

Control Union Certification BV., has performed an inspection, as mentioned in Reg. (EC) 834/2007 repealed by Reg. (EU) 2018/848; Reg. (EC) 889/2007 repealed by Reg. (EU) No 1165/2021, assigned by:

Name of licensee	: Wise Use International B.V
Address	: Zeemanlaan 7, : 3401 MV Ijsselstein, The Netherlands.
Legally represented by	: Mr. De Heer Wisman
Authorized	: Mr. De Heer Wisman
Country	: The Netherlands.

This approval referred to in the licensee contract, covers the following product(s). The approval of additional inputs for organic processing/production by Control Union Certification BV., is based on the criteria mentioned in Reg. (EC) 834/2007 repealed by Reg. (EU) 2018/848; Reg. (EC) 889/2007 repealed by Reg. (EU) No 1165/2021 Regulations.

Product no.	Name of product	Product category	Use for	Processing unit no.	Product Status
800845- P01	B Leafz	Fertilizer	Nutrient	PRC 000975	Allowed
800845- P02	B Rootz	Fertilizer	Nutrient	PRC 000975	Allowed
800845- P03	NaMa Balance	Fertilizer	Nutrient	PRC 000975	Allowed
800845- P04	P en R	Fertilizer	Nutrient	PRC 000975	Allowed
800845- P05	Poco	Fertilizer	Nutrient	PRC 000975	Allowed
800845- P06	Tricoamin TH BS und Prosubstrat TA	Fertilizer	Nutrient	PRC 000975	Allowed
800845- P07	DWA	Feed Additive	Feed Additive	PRC 000975	Allowed

CONTROL UNION

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Annex to Statement of Compliance

APPROVAL NO°: CU 800845 EC-INP-01-2025

This approval covers the following **input manufacturing unit(s)** which meets the criteria of Reg. (EC) 834/2007 repealed by Reg. (EU) 2018/848; Reg. (EC) 889/2007 repealed by Reg. (EU) No 1165/2021 including the amending regulations:

Unit no.	Name of Unit	Address	Process
PRC 000975	Wise Use International	Zeemanlaan 7 Ijsselstein, The Netherlands	Export, Administration Mixing, Storage.

This approval including the annex remains property of Control Union Certification BV., and can be withdrawn in case of termination as mentioned in the licensee contract, or in case changes or deviations of the above-mentioned data occur. The licensee is obliged to inform CU immediately of any changes in the above-mentioned data. Only an original and signed approval is valid.

This confirmation by Control Union is not an endorsement of the product(s) and does not guarantee the efficacy of the product(s) or the compliance to any statutory requirement.

Date: 14/02/2025

Authenticated by:
on behalf of the Managing Director

Ranjitha KS
Control Union Certification BV

NURTOCORP BV Certificate GMP+ FSA -Feed Safety Assurance

CERTIFICATE COPY

CERTIFICATE No: C 893489GMP-02.2023
REGISTRATION No: CU 893489
GMP+ registration no: GMP058028

Field of attention:

GMP+ Feed Safety Assurance

Issued to:

Nutrocorp International B.V.

Zeemanlaan 7
3401 MV Ijsselstein
Nederland

Control Union Certifications B.V., registration no: CI000032, declares that there is justifiable confidence that the GMP+ scope(s), as specified in the Annex, at the above mentioned GMP+ Certified Company complies with the applicable requirements and conditions of the GMP+ Feed Safety Assurance Module 2020

Trade in feed

Valid until: 06 March 2026

Date of certification: 07 March 2023
Place and date of issue:
Zwolle, 10 March 2023

Declared by:

On behalf of the Managing Director

Mrs. J. Bontan

Certifier
Control Union Certifications B.V.
Meeuwenlaan 4-6
8011 BZ ZWOLLE
The Netherlands

i/o

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Annex for GMP+ CERTIFICATE

COPY

Name of licensee	Nutrocorp International B.V.
Address	Zeemanlaan 7 3401 MV Ijsselstein Nederland

The scope of this certificate relates to:

Trade in feed

Specified activities / animal feeds :

Compound feed

This certificate including the annex remains property of Control Union Certifications B.V. and can be withdrawn in case of terminations as mentioned in the licensee contract, or in case changes or deviations of the above mentioned data occur. The licensee is obliged to inform Control Union Certifications B.V. immediately of any changes in the above mentioned data. Only an original and signed certificate is valid.

Place and date of issue:
Zwolle, 10 March 2023

Mrs. J. Bontan

Certifier

i/o

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CERTIFICAAT

CERTIFICAAT Nr: C 893489GMP-01.2023
REGISTRATIE NR.: CU 893489
GMP+ registratie nr.: GMP058028

Toepassingsgebied:

GMP+ Feed Safety Assurance

Uitgereikt aan:

Nutrocorp International B.V.

Zeemanlaan 7
3401 MV Ijsselstein
Nederland

Control Union Certifications B.V., registratie no: CI000032,verklaart dat er een gerechtvaardigd vertrouwen bestaat dat de GMP+ scope(s), gespecificeerd in de Annex, bij het bovengenoemde GMP+ gecertificeerde bedrijf voldoen aan de geldende eisen en voorwaarden van de GMP+ Feed Safety Assurance Module 2020.

Handel in diervoeder

Dit certificaat blijft van kracht tot nadere aankondiging, vooropgezet dat de bovengenoemde deelnemer blijft voldoen aan de eisen zoals vastgelegd in de overeenkomst met Control Union Certifications B.V. Op grond van de jaarlijkse audits, uitgevoerd door Control Union Certifications B.V., is dit certificaat:

Geldig tot: 06 Maart 2026

Datum certificering: 07 Maart 2023
Plaats en datum van afgifte:
Zwolle, 07 Maart 2023

Aldus verklaard:

Namens de directeur

Mrs. J. Bontan

Certificeerder
Control Union Certifications B.V.
Meeuwenlaan 4-6
8011 BZ ZWOLLE
The Netherlands

i/o

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Annex van GMP+ CERTIFICAAT

Licensiehouder	Nutrocorp International B.V.
Adres	Zeemanlaan 7 3401 MV Ijsselstein Nederland

De scope van dit certificaat heeft betrekking op:

Handel in diervoeder

Gespecificeerde producten/diensten :

Mengvoeders

Dit certificaat inclusief de bijlage blijft eigendom van Control Union Certifications B.V. en kan ingetrokken worden in geval van beeindiging zoals genoemd in de licentie-overeenkomst of in geval wijzigingen of afwijkingen van de hierboven genoemde gegevens optreden. De licentiehouder is verplicht Control Union Certifications B.V. direct te informeren over wijzigingen van de genoemde gegevens. Alleen een origineel en ondertekend certificaat met bijbehorende bijlagen is geldig.

Plaats en datum van afgifte:
Zwolle, 07 Maart 2023

Mrs. J. Bontan

Certificeerder

i/o

CONTROL UNION CERTIFIED

Registration Netherlands Food and Consumer Product Safety Authority -Ministry of Agriculture, Fisheries, Food Security and Nature

Nederlandse Voedsel- en Warenautoriteit
Ministerie van Landbouw, Natuur en Voedselkwaliteit

> Retouradres Postbus 43006 43006 3540 AA Utrecht

NutroCorp International B.V.
Zeemanlaan 7
3401 MV IJsselstein Ut

directie Handhaven
divisie Klant, bedrijf en consument

afdeling Klantbestand & verleningenbeheer
Catharijnesingel 59
3511 GG Utrecht
Postbus 43006
3540 AA Utrecht
www.nvwa.nl

Contactpersoon
afdeling Klantbestand & verleningenbeheer

T 0900 03 88
F 088 223 33 34
info@nvwa.nl

Onze referentie
NVWA/GW21199

Datum 09 februari 2023
Betreft Actueel overzicht

Geachte heer, mevrouw,

Het onderstaande overzicht toont de actuele gegevens van uw organisatie zoals deze nu bij ons bekend zijn.

KvK-nummer:
88733335

Bedrijfsnaam:
NutroCorp International B.V.

Vestigingsadres:
Zeemanlaan 7
3401 MV IJsselstein Ut

Postadres:
Zeemanlaan 7
3401 MV IJsselstein Ut

Verleningsobject(en):

Nr.	Vestigingsnr.	Omschrijving	Adres	Postcode	Plaats
224930	000054574293	NutroCorp International B.V.	Zeemanlaan 7	3401 MV	IJsselstein Ut

Doen zich in de toekomst wijzigingen voor in uw gegevens? Geef dit dan tijdig door aan de NVWA.
U bent dat wettelijk verplicht. Op www.nvwa.nl in zoek op: Een wijziging doorgeven leest u hoe u dat kunt doen.

Hoogachtend,

Pagina 1 van 2

directie Handhaven
divisie Klant, bedrijf en consument

Datum
09 februari 2023

Onze referentie
NVWA/GW21199

Dr. Ir. C. Vroliik
divisiehoofd Klant, bedrijf en consument

Nederlandse Voedsel- en Warenautoriteit
Ministerie van Landbouw, Natuur en Voedselkwaliteit

> Retouradres Postbus 43006 43006 3540 AA Utrecht

NutroCorp International B.V.
Zeemanlaan 7
3401 MV IJSSELSTEIN UT

directie Handhaven
divisie Klant, bedrijf en consument

afdeling Klantbestand & verleningenbeheer
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3511 GG Utrecht
Postbus 43006
3540 AA Utrecht
www.nvwa.nl

Contactpersoon
afdeling Klantbestand & verleningenbeheer

T 0900 03 88
F 088 223 33 34
info@nvwa.nl

Onze referentie
NVWA/1071503.01

Datum 09 februari 2023
Betreft Registratie

Geachte heer, mevrouw,

U heeft zich bij de Nederlandse Voedsel- en Warenautoriteit aangemeld voor een diervoeder registratie.

Met betrekking tot deze melding bericht ik u als volgt:

Wij hebben uw inrichting NutroCorp International B.V. aan het adres Zeemanlaan 7 te IJsselstein Ut met ingang van 09 februari 2023 geregistreerd voor **de handel in aanvullende en/of volledige diervoeders voor voedselproducerende dieren (TRADE FEED FPA).**
Uw registratienummer is NL 224930.

Registratie is op grond van artikel 9 van Verordening (EG) nr. 183/2005.

Hoogachtend,
DE MINISTER VAN LANDBOUW, NATUUR EN VOEDSELKWALITEIT,
Voor deze:
HET DIVISIEHOOFD KLANT, BEDRIJF & CONSUMENT VAN DE DIRECTIE HANDHAVEN VAN DE NEDERLANDSE VOEDSEL- EN WARENAUTORITEIT

Dr. Ir. C. Vrolijk
divisiehoofd Klant, bedrijf en consument

BEZWAARMOGELIJKHEID
Als u het niet eens bent met dit besluit, kunt u binnen zes weken - na verzending van dit besluit -een bezwaarschrift indienen. Doe dit op tijd, anders kan uw bezwaarschrift niet worden behandeld. Let wel: het indienen van een bezwaarschrift schort de werking van het besluit niet op.

Bij voorkeur kunt u uw bezwaarschrift via de e-mail verzenden naar nvwabezwaarenberoep@nvwa.nl. Uw bezwaarschrift kunt u eventueel ook per

BIOZAR ORIGINAL BV CERTIFICATIONS

CERTIFICATE

The Food Safety Management System of

Biozar Original BV

at

Zeelmanlaan 7 , 3401 MV IJsselstein, Netherlands

has been assessed and determined to comply with the requirements of

ISO 22000:2018

Control Union Certifications declares to have inspected the unit(s), and/or product(s) of the above-mentioned client, and have found them in accordance with the standards mentioned above.

This certificate is applicable for the scope of:

Trading of nutritional supplements.

Food Chain Subcategory: FII

Certificate number:	**C884645SO22000-01.2025**
Registration number:	**CU884645**
Certification decision date:	**07/05/2025**
Initial certification date:	**09/05/2022**
Certificate issue date:	**07/05/2025**
Valid until:	**08/05/2028**

CSmith

Cindy-Lee Smith,
Certifier

Authorised by, on behalf of Control Union Certifications B.V.

MGMT. SYS.
RvA C 499

CONTROL UNION CERTIFIED
ISO22000

CONTROLUNION

Issue by: Control Union Certifications B.V. P.O. Box 161, 8000 AD, Zwolle, The Netherlands. Tel: +31 0384260100
To verify this certificate please go to http://cucpublications.controlunion.com/certified_companies_and_products.aspx
This certificate remains the property of Control Union Certifications B.V. and remains in force until further notice, provided that the above-mentioned client remains in compliance with the requirements as laid down in the contract with Control Union Certifications and based on annual audit performance.

ADDENDUM to CERTIFICATE No: C884645SO22000-01.2025
REGISTRATION No: CU884645

ISO 22000:2018

Name Company: **Biozar Original BV**
Legally represented by: **Mrs Duijts- Wisman**
Following Processing unit(s) and Process(es) is(are) covered under the Scope of Certification:

Unit no.	Name of unit	Unit ref.	Address	Processes
	Biozar Original BV		Zeelmanlaan 7 , 3401 MV IJsselstein, Netherlands	FII - Trading of nutritional supplements.

This certificate is issued by Control Union Certifications B.V. and remains property of Control Union certifications. This certificate can be withdrawn in case of terminations as mentioned in the licensee contract, or in case changes or deviations of the above-mentioned data occur. The licensee is obliged to inform Control Union Certifications immediately of any changes in the above-mentioned data. Only an original and signed certificate is valid.

Certification decision date: **07/05/2025**

Initial Certification date: **09/05/2022**
Certificate issue date: **07/05/2025**
Valid until: **08/05/2028**

CSmith

Cindy-Lee Smith

Authorised by, on behalf of
Control Union Certifications B.V.

Issue by: Control Union Certifications B.V. P.O. Box 161, 8000 AD, Zwolle, The Netherlands.
Tel: +31 0384260100 http://www.controlunion.com

STATEMENT OF COMPLIANCE

REGISTRATION NO°: CU 865420

Control Union Certification BV., hereby approves the input manufacturing done by

BAC Agro International B.V.
Bleiswijk, The Netherlands

Project in: The Netherlands

on the basis of inspections done at the input manufacturing unit. This approval is valid for those product(s) and area(s) that are specified in the annex. The validity of this approval solely depends on the continued compliance with the Reg. (EC) 834/2007 repealed by Reg. (EU) 2018/848; Reg. (EC) 889/2007 repealed by Reg. (EU) No 1165/2021 and is subject to surveillance inspections.

Valid until: 14th February 2026

This approval is in force until further notice, provided that the above-mentioned licensee continues meeting the conditions as laid down in the licensee contract with CU. Based on the annual inspections that CU performs, this approval is updated and kept in force.

CONTROLUNION

Date of approval
15/04/2025

Declared by:
on behalf of the Managing Director

Ranjitha KS
Control Union Certification BV

Annex to Statement of Compliance

APPROVAL NO°: CU 865420 EC-INP-02-2025

Control Union Certification BV., has performed an inspection, as mentioned in Reg. (EC) 834/2007 repealed by Reg. (EU) 2018/848; Reg. (EC) 889/2007 repealed by Reg. (EU) No 1165/2021, assigned by:

Name of licensee : BAC Agro International B.V.
Address : Spectrumlaan 39, 2665 NM Bleiswijk, The Netherlands
Legally represented by : Mr. Sander Roxs
Authorized : Mr. Sander Roxs
Country : The Netherlands

This approval referred to in the licensee contract, covers the following product(s). The approval of additional inputs for organic processing/production by Control Union Certification BV., is based on the criteria mentioned in Reg. (EC) 834/2007 repealed by Reg. (EU) 2018/848; Reg. (EC) 889/2007 repealed by Reg. (EU) No 1165/2021 Regulations.

Product no.	Name of product	Product category	Use for	Processing unit no.	Product Status
865420- P01	BAC Bio Granulaat	Fertilizer	Nutrient	PRC 103827	Allowed
865420- P02	Bio Rootz	Fertilizer	Nutrient	PRC 103827	Allowed
865420- P03	BAC Fulvic	Fertilizer	Nutrient	PRC 103827	Allowed
865420- P04	Organic Bloom	Fertilizer	Nutrient	PRC 103827	Allowed
865420- P05	Organic Grow	Fertilizer	Nutrient	PRC 103827	Allowed
865420- P06	Organic PK Booster	Fertilizer	Nutrient	PRC 103827	Allowed
865420- P07	ProBalance	Fertilizer	Nutrient	PRC 103827	Allowed
865420- P08	Bio-Seed Red	Fertilizer	Nutrient	PRC 103827	Allowed
865420- P09	OPF (mix Grow/Bloom)	Fertilizer	Nutrient	PRC 103827	Allowed
865420- P10	Bio-Seed	Fertilizer	Nutrient	PRC 103827	Allowed

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Annex to Statement of Compliance

APPROVAL NO°: CU 865420 EC-INP-02-2025

This approval covers the following **input manufacturing unit(s)** which meets the criteria of Reg. (EC) 834/2007 repealed by Reg. (EU) 2018/848; Reg. (EC) 889/2007 repealed by Reg. (EU) No 1165/2021 including the amending regulations:

Unit no.	Name of Unit	Address	Process
PRC 103827	BAC Agro International B.V.	Spectrumlaan 39 Bleiswijk, The Nederlands	Filling, Mixing, Storage, Sales

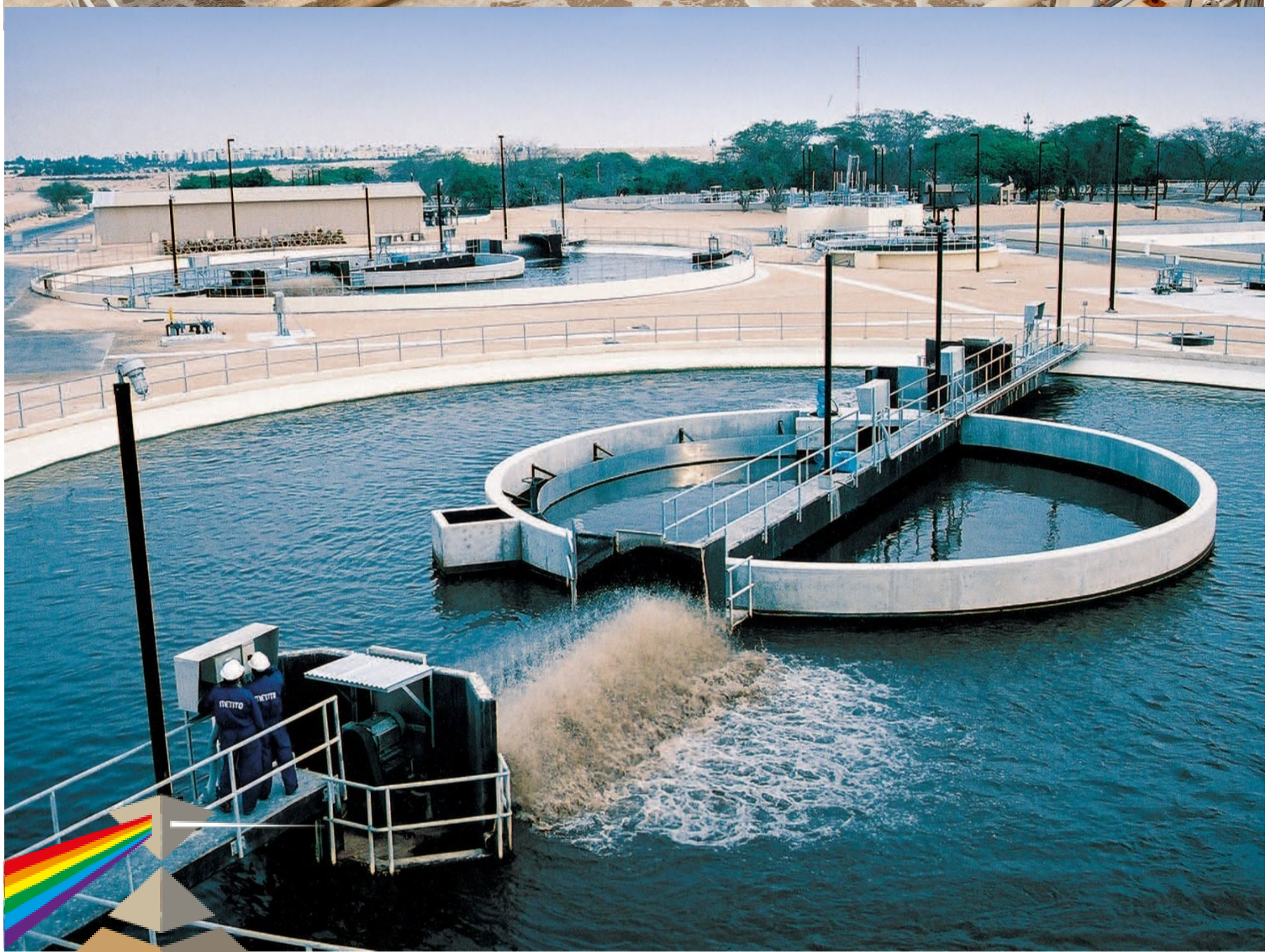
This approval including the annex remains property of Control Union Certification BV. and can be withdrawn in case of termination as mentioned in the licensee contract, or in case changes or deviations of the above-mentioned data occur. The licensee is obliged to inform CU immediately of any changes in the above-mentioned data. Only an original and signed approval is valid.

This confirmation by Control Union is not an endorsement of the product(s) and does not guarantee the efficacy of the product(s) or the compliance to any statutory requirement.

Date: 15/04/2025

Authenticated by:
On behalf of the Managing Director

Ranjitha R S
Control Union Certification BV



WISEUSE
INTERNATIONAL

The Biochemical Clean Up of the Polluted Lake Ypacarai, Paraguay
Final Report of the Feasibility study
October 2008

PESP- 07038 Paraguay

Prepared by:
Wise Use International BV
Trasmolenlaan 8D
3447 GZ Woerden
The Netherlands

Feasibility study PESP- 07038 Paraguay
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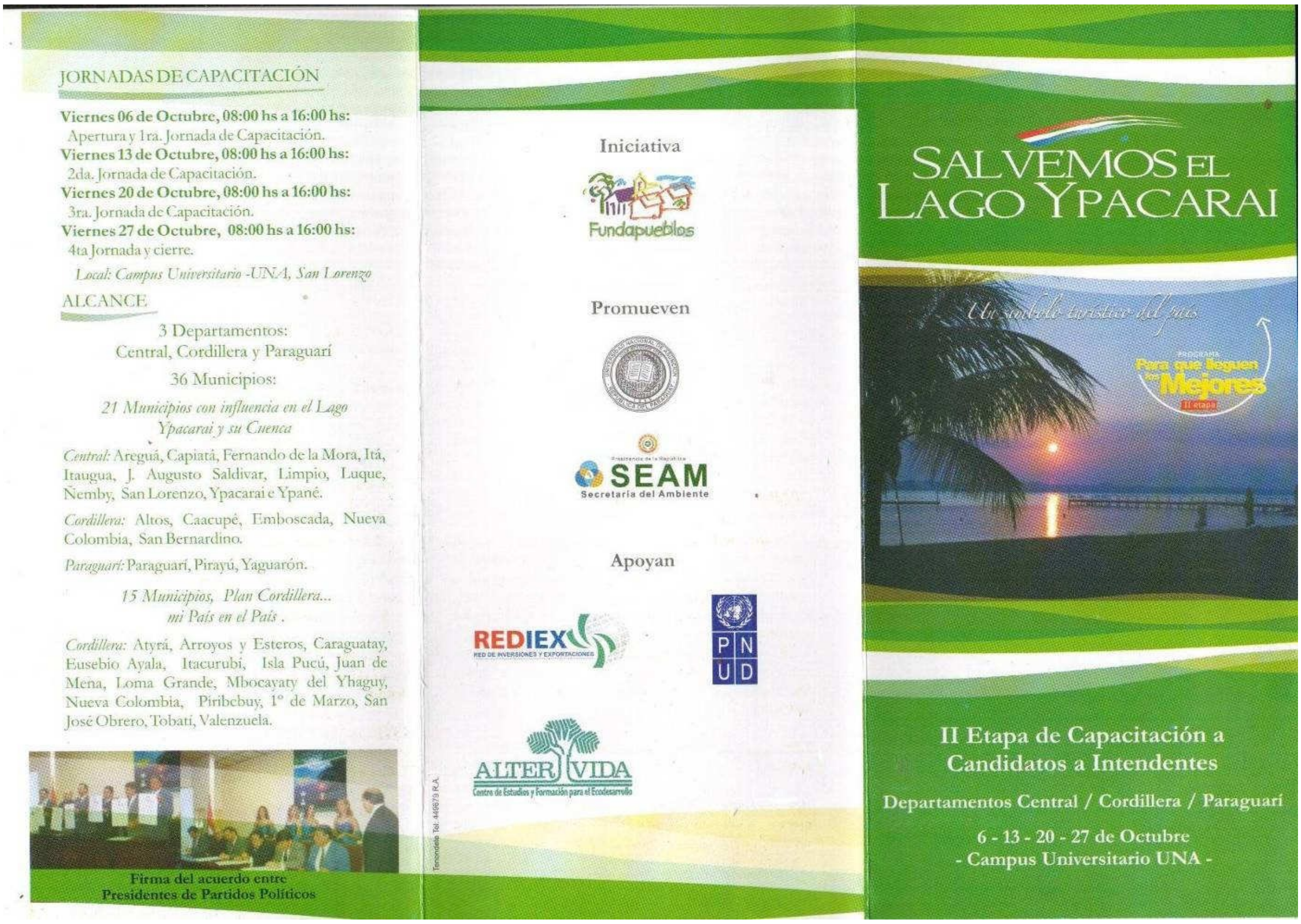
2. Proposal University of Padova / Venezia hydrographic Basin, Prof. S. Fattorelli (2007)
3. M.O.U. presidential candidates compromising the cleanup of the Lake (2008)
4. Preliminary study and laboratory tests, carried out by Wise Use (India), REDIEX and CEMIT.
5. Final report, conclusions and recommendations of CEMIT, University of Asunción, Paraguay, on the tests in the Ypacarai Lake. Both the spanish original and its official english translation.
6. Report WUR, as they decided to send their conclusions directly to EVD, we just attach their working plan.
7. Commercial offers Handels & Adviesbureau Visser
8. Commercial offers Wise Use International
9. Touristic Information (statistics of SENATUR) of Paraguay
10. Copy of Environmental Laws; LEY 369, 836 (SEAM)

Boxes

Figures

Tables



EXECUTIVE SUMMARY

The Ypacarai hydrographic basin, being close to the capital Asuncion of Paraguay suffers from environmental problems, due to decades of uncontrolled discharge of industrial and domestic pollutants. The hydrographic basin includes the Ypacarai lake, about 12 x 5 km in size, a natural lake but shallow due to the catchment of sediments originating from the bordering areas. By means of various hydraulic controls the authorities have tried to maintain a sufficient water level in the lake. Some of these measures however resulted in the opposite.

Climatically Paraguay is subtropical, landlocked and has very hot dry summers. Evaporation is high during this period. At the inlets and outlet of the lake wetlands are present, whose sizes matches still both the lake size and the main discharges of the “quebradas” (streams). The increased pollution has led to various environmental and economic “disasters”: a lake bottom (biofilm) with almost no biological interaction, polluted wetlands with little phyt-purification possibilities, no more fishery for the surrounding (most indian) villages, high costs involved when used for intake of drinking water.

The Ypacarai
lake, once the national pride, is really the only lake of size (60 km2) and importance in Paraguay. Many important hotels and other touristic attractions surrounding the lake are now closed and swimming forms a real threat to health.

The Ministries of Commerce, of Environment (SEAM) and of Public Works (MOPC), leaded by the MOPC, tried to initiate feasibility studies to clean the lake, in order to, establish a healthy tourist and economic environment. Some (international) proposals, for instance the dredging by diskcutter suction have been made, but all rejected by the very high costs involved.

The Paraguayan Government has proclaimed that it is in their
greatest interest to be able to clean the polluted lake and wetlands and prevent a further pollution influx. This M.O.U was signed by all 4 presidential candidates, before the election took place (april 2008); which means that the new government (that assumed power in august 2008) will do his utmost to regain an environmantal healthy state of the lake.

In view of the above stated the agreement between Wise Use int. and the MOPC (acting as leading ministry) was signed and a Dutch contribution received under the PESP programme (with date 12/10/2007) to conduct the feasibility study to investigate the functionality of biocatalyst in the Ypacarai lake.

The investigation comprises an in-situ part
(within the lake) and several laboratory analysis. The scientific part to be carried out by CEMIT, department of biochemics, University of Asunción and Wageningen University, Aquatic Ecology and Water Quality Management group (AEW).

Cooperation between all parties involved started smoothly and a first mission to Paraguay, including a scientist from Wageningen (WUR) started end October 2007. Lake and lake bottom samples were taken and brought to the CEMIT laboratories for examination. A dispute between Wise Use and WUR about the in-situ test procedures and the payment procedure (although well regulated by PESP) arose. CEMIT fully backed Wise Use but the responsible scientist from AEW unofficially refused further cooperation for in-situ analysis . WiseUse did sent many samples from the lake, taken during the in situ-tests, plus specimen of the biocatalyst to the Wageningen laboratories for analysis.

This analysis apparently has been carried out, the official results are not (yet) presented to Wise Use,
and thus cannot be included in this final report. Unofficially the AEW informed Wise Use that, because of the protocol/ procedural disagreements with Wise Use, their results on samples sent from Paraguay to their laboratories, will be sent to the EVD directly. 7
However, entrusting the CEMIT capability of sampling and analysis (which by the way was confirmed by the AEW during their first and only visit) this report present their biochemical findings in Annex, forming an essential part of the feasibility study.

The Wise Use team and a MOPC representative have already paid visits to the main international financial institution present in Paraguay (IADB). Its president has assured that the bank is very interested, when the Paraguayan State officialises the present feasibility results as being positive and decides to continue with the Project Preparation for a biochemical cleanup of the lake and wetlands plus preventive measures (WWTP's).
The feasibility study took place in several stages, a first pre-PESP study on the biochemical analysis of lake samples and Lake Bottom material (carried out by CEMIT).
This was followed in October 2007 by first sonar and sampling analysis by AEW (Wageningen).

The in-situ testing with the biocatalyst started after the main summer holidays in Paraguay, thus in march 2008. Test procedures were written and a large pool, adjacent and connected to the lake was closed off, followed by the introduction of the biocatalyst and monitoring the biochemical parameters on a daily (and later on a weekly) basis. The testing perios ended in May 2008, after which the remaining biochemical laboratory test had to be performed. The results of CEMIT were officially presented to WiseUse in the end of October 2008 and annexed to this report.

Some of the CEMIT conclusions are stated here:
- A substantial decrease in total coliforms has been observed after supplying the Biocatalyst
- In all subsequent stages of the treatment a remarkable decrease in the cyanobacteria population was observed, which is very important as the main problem of the lake is its state of europhization.
- The zooplancton population, specifically the copépodos, increased due to the major biodiversity of nutrients caused by the decrease of cyanobacteria and increase of other divisions.

Study objective
This report presents the feasibility study for the biological cleaning of the highly polluted Ypacarai Lake in Paraguay. The applicants –Wise Use and Handelsburo Visser- are companies with a well established track record in bio-technology applied across various sectors (environment, horticulture, ethanol production). The local client is the "Ministerio de Obras Publicas y Comunicaciones (M.O.P.C.)", Paraguay.

Field work and testing
The in-situ field tests, executed by the Univ. of Asunción, CEMIT with logistical assistance of the Ministry of Public Works and co-funded by the Min. of Economic Affairs of the Netherlands (EVD) are very important and valuable, as they have proved the value of the biocatalyst in obtaining a significant increase towards a more healthy Ypacarai hydrographic basin.

The fieldwork comprised all preparations, hardware, importing biocatalyst from Holland, the first sonar measurements and Lake Bottom samples by WUR and of course all testing on a daily basis both in the lake water and the laboratory.
Wise Use also arranged for some pumps to bring more circulation in the pool during testing and provided his biocatalyst.

The value of these tests (the results) are important as
it could provide the Paraguayan State with alternatives for cleanup and preventive measures next to very costly (dredging) operations. The results if accepted to be positive may help the Government of Paraguay in obtaining funds to execute the cleanup and the preventive (hardware0 .measures. The total CAPEX of the proposed solution amount to 34.2 million Euros which is many times less than dredging operations (which also do not have any preventive components).

Proposed solution

As awaiting fully compliance with the existing environmental laws (where regulations still have to be prepared and politically accepted) is just still impossible due to non-existing sanitation infrastructure in the Hydrographic basin of Ypacarai. Meanwhile other mitigating measures have to be taken. Awareness is one, but a “clean” healthy and balanced lake is the other. Dredging the lake bottom is a possibility but cost wise not feasible.
This is the reason that biochemical solutions are sought.
The present feasibility testing, co-funded under the PESP-program by the Dutch Government, is therefore of vital importance. Not only is focused on the lake and wetland solution itself but of course also on prevention.
The Paraguayan authorities and NGO’s present in the area have also insisted on the formulation of prevention measures. It became quite clear during the PESP-feasibility study that the prevention problem is huge, and thus Wise Use focused on an intermediate wastewater treatment plant with the addition of the biocatalyst, located at the end of the Yuquyry stream (the most polluting stream entering the lake), just before the stream enters the wetlands.

According to the CEMIT laboratory tests this bio-boost will have its immediate effect on the polluted water that enters the wetlands and then the Ypacarai lake.

Literature review and discussions with Paraguayan biochemists suggest this is a good method for now to be implemented in this stage of the total solution.
The lake water and especially the bottom itself may also be treated with the biocatalyst. Suggestions and
financial indications have been reported to the authorities during meetings. The solution (also according to the available literature) is simple: The most important is prevention: try to diminish the inflow of nutrients and find a solution to clean the lake bottom (e.g by biocatalysts). Then the ecological balance will be restored. 9

Acknowledgements

The executive team for this PESP funded program wishes to thank the following people in Paraguay for all things they have accomplished and their struggles to get this test program done. The team recognizes the fact that politics sometimes have a large influence in all matters that concern the tasks to be performed, both in time and effort needed. Next to ***all ministries involved in Paraguay***, including the Ministers and Vice-ministers (past and present) special thanks go to:

Prof. Ing. Luis Alberto Meyer (who passed away in august 2007), former Minister of Planification and initiator of the Ypacarai cleanup Program. Without his never ending efforts (especially the political drive) this program never would have started anyhow
Dr. Derlis Esteche, founder and director of the Fundapueblos foundation and former minister of Tourism. His foundation is of the utmost importance for awareness of the population and thus a (future) sustainable healthy environment. He has managed to get all 4 presidential candidates to sign a M.O.U. in which the elected President compromises himself to do his best in stimulating the cleanup of the Lake and wetlands and the prevention part (sewerage and treatment).

Ing. (arch.) Luis D. Añazco Franco, de la "Dirección de Obras Publicas (M.O.P.C.)" who was always present, organized meetings, arranged all logistics during the in-situ tests and who was a person of the MOPC we always could ask if something unexpected arose.
The ***rectorate of the University of Asunción*** and ***Prof. Dra. Inocentia Peralta*** CEMIT.
She in her enthusiasm was always willing to discuss the bio-matters; the analyses, the protocols and the results.

Ing. Rubén Dumot, president of the Club Nautica at the lake side of the Ypacarai Lake, who gave permission to use the pools at the Club for the in-situ tests and delivered all energy /boats for free.

The ***Embassy of the Kingdom of the Netherlands*** for Uruguay and Paraguay and the Dutch consulate in Paraguay. The positive answer from the Embassy to support this feasibility study helped the team to continue their efforts, both the administrative and the technical part.
The ***PESP- team of the EVD*** / Min. of Econ. Affairs of The Netherlands, with healthy criticism and support, and always open minded.

10. 1 INTRODUCTION

This report presents the feasibility study for the biological cleaning of the highly polluted Ypacarai Lake in Paraguay. The applicants –Wise Use and Handelsbureau Visser- are companies with a well established track record in bio-technology applied across various sectors (Environment, Eco-Friendly Pollution Control Technologies, Horticulture, Stimulators for Alcohol Fermentation Processes, Animal Husbandry etc).

The local (Paraguayan) leading counterpart consists of the "Ministerio de Obras Publicas y Comunicaciones (M.O.P.C.)". This Ministry thas been appointed by the Paraguayan Government to act as leading ministry for both the cleanup of the lake and the implementation of civil engineering and hydraulic works to prevent a further inflow of pollutants. The lake is highly polluted by more than 30 years uncontrolled influx of merely industrial and urban wastewater.

Depositions of sediments during geological times and mistakes in hydraulic designs have also contributed to the decrease in depth of the Lake Ypacarai. Both factors have resulted in an increasing biological unbalance of the lake water and its (sub)bottom; and as such it cannot safely be used anymore for touristic purposes and for intake of drinking water and fishery for the surrounding (most indian) villages. The Lake Ypacarai, once the national pride, is the only lake of size (60 km2) and importance in Paraguay. Now all important hotels surrounding the lake are closed and swimming forms a real threat to health.

The Ministries of Commerce, of Environment and of Public Works (MOPC), as stated leaded by the MOPC, now have tried to initiate the feasibility studies to clean the lake, in order to, once again, establish a healthy tourist and economic environment. Some (international) proposals have been made, but all rejected due to high costs and non feasible technologies involved. The Paraguayan Government has proclaimed that it is, next to the prohibition of the inlet of polluted water, in their greatest interest to be able to clean the polluted lake itself, preferably on a biological/biochemical basis at an acceptable cost.

11.2 INTRODUCTION OF THE CONSORTIUM AND THE LOCAL COUNTERPART

***2.1* THE DUTCH CONSORTIUM**

The Dutch Consortium consists of the following firms:
- Wise Use International b.v., and Handelsburo Visser b.v.

The Dutch consultants are:
- Centre for Water and Climate of Wageningen University; the Aquatic Ecology and Water Quality Management group.
- ing. Laurens Trebes (TCA Consult),
- dr. ir. Marinus Pool (Geonamic)
- dr. Ronny Venegas Carbonell.

WiseUse Int. b.v. (www.wiseuse.nl)
Role in the PESP study:
- **Contract partner with the EVD and first applicant**
- **Application of bio-technology and supplier of future equipment and supplies**
- **Supplier of Biological activators for micro-organisms.**

Being active for almost 10 years in the Netherlands, Germany, England and Spain, it has formed recently Joint Ventures in the bio-technology sector in India, Ireland en the United States.

The company has developed a wide range of biological products, applicable for Waste

Water Treatment, Solid Waste Treatment, Bio-Composting, Bio-Methanation, Alcohol Fermentation, Food Processing, Horticulture and the Flora Industry. All the Products are 100% Bio-Degradable Pure Natural Products. At a large scale for water treatment, food processing, horticulture and the flora industry.

Their products are 100% biological and as such completely degradable.
The biological products are certified by Control Union (earlier named SKAL) and as such may also be used for food preparation processes for human consumption. These products act as Organic Cell-Stimulators and do not contain any type of enzymes, special bacterial composites or hormones. The sister company Bio Stimulators Ltd (Bangalore, India) of WiseUse Int. BV is involved in project development and the search for biotechnological solutions for environmental problems, as e.g. the cleaning of polluted lakes in India and the here presented Lake Ypacarai in Paraguay. A team of specialists (biotechnologists and engineers) is actively working worldwide. The products are multipurpose and applicable for upcoming advanced Bio-Technology and Microbiology Sectors

Product overview:
• PoCo (Pollution Control): It acts as an Organic Cell-Stimulators which accelerates rate of growth of micro-organisms in a natural way by supplementing micro-nutrients and trace elements which helps to enhances the rate of Microbial and Bio-Chemical Reactions. In nutshell PoCo is an eco-friendly specific tool for problems related to Environment and Waste Treatment Technologies. It helps for faster growth of Biomass, Maximum reduction of BOD/COD levels, faster degradation of Organic Matter, Reduction of Foul Smelling within specific time etc and many more. It has been widely report to clean Lagoons, Ponds, Small Rivers, Lakes with efficient good results and same has been acknowledged here within. 12
• BFR (Bio-Fermentor): It is a dark brown viscous liquid having eukalyptus smell and is a combination of Plant Extracts and Fruit Oil - a Pure Natural Product. It is 100% Bio Degradable and acts as an Organic Cell-Stimulators which enhances the rate of Microbiological and Bio-Chemical Reactions of the yeast during Fermentation Process. It helps to achieve Higher Levels of Alcohol along with Superior Quality of Final Product with a minimum of impurities and also helps to reduce considerably the Fermentation Time.

Handels en Adviesburo Visser b.v.
Role in the PEPS study:
- **supplier of technical equipment**
- **supplier of engineering knowledge**

Visser Handelbureau was founded in 2004 and is an advisory for firms using special cultivation techniques for greenhouse products, water treatment and fermentation processes.

In collaboration with WiseUse International the firm has developed low cost mechanical equipment for niche markets.
Some recent developments comprise (in Dutch):

1. Special Dosing Units
2. JUMBO tank and aeration pumps
3. Aeration plates for water treatment plants
4. Packaging for bio catalysts
5. Lavafilters for water treatment plants
6. Aquarium equipement
7. Tanks (inox)

The director/owner of the firm has build up a long experience in the greenhouse cultivation. After 2004 the main activities of the firm shifted towards hardware applications in the various industries Freelance workers with special knowledge are contracted to ensure the design and construction of several new developments. Only ordered and special designed products are manufactured. An intense cooperation with other experienced firms results in innovative installations and machinery, especially within the sector of (waste) water treatment. The economic situation
of the greenhouse industry in the Netherlands and their specific knowledge, among other factors, helped a lot to introduce themselves to the international markets.
TCA (www.tcaconsult.com)

Role in the PESP study:

• Project support in Paraguay
• Design of the test facilities
• Contacts with local suppliers and local banks

TCA started its activities in 1992 in Holland and expanded the project in1997 with opening an office in Venezuela.

Office: Venezuela
TCA started in Venezuela an initiative to offer a special service to Dutch companies that are interested in starting business in Latin America.
Activities:

• Partner searches 13
• Market Studies
• Feasibility Studies
• Organization of trade missions
• Accompanying of executives
• Scouting/sourcing

In a short time span business started to grow. In Chile, Argentina, Uruguay and Brazil the company started to cooperate with agents who provided us with information according the inquiries of our clients. The network of agents is still growing. Recently in 2006 we started cooperation with companies and independent persons in Panama, Paraguay and Costa Rica.

For more than 10 years TCA has a joint partnership with the NCH (Nederlands Centrum voor Handelsbevordering, Den Haag). TCA acts as their correspondent and organize special activities to improve Dutch – Latin American business. Also in cooperation with embassies of different countries some market studies, trade fairs and matchmaking programmes are executed.

NMCP

At the same time TCA volunteered for the NMCP (Netherlands Management Consultants Programme), better known in Holland as PUM; a governmental non profit organization that sends out retired executives to help starting up projects in developing countries. Venezuela at that time was a developing country and we achieved the first year about ten project start-ups in several fields. Nowadays Venezuela can not longer apply for participation in the programme.

Office: Caracas, Venezuela

Recently, October 2006 TCA was contracted to carry out the Match-making activities for more than 22 Dutch companies who visited Venezuela with an EVD trade mission. In February and March TCA was active in Panama for the EVD again (the programme 2g@there). TCA organized the trade mission and the match making programme, build up the Dutch Pavilion and accompanied Dutch participants. TCA is currently in the process of the execution of a PESP project for Dalsem (Venezuela, PESP 06075) and Kiremko (PESP 07025) These project are in progress and it is a fact that the first contracts for greenhouses with the Venezuelan Ministry of Agriculture will be signed in the third quarter of 2008. Furthermore TCA is contracted bij NCH to head an incoming mission from Venezuela to the Netherlands, may 2008.

Geonamic (dr.ir. M.A. Pool).

Role in the PESP study:

• Project Coordination

• Contacts with local and federal governments

• Contacts with Universities of Wageningen and Asunción

Experienced as a hydrologist, geologist and having performed various international marketing studies, dr. ir. Marinus Pool took part in several special missions, mostly to Latin America. Recently he was head of a long term Expert Mission to Venezuela for Disaster Prevention (intended project time 5 years), signed between the European Commision and the Bolivarian Republic of Venezuela. Before this he was for a long time attached to the Marketing and Developing Department of Royal Boskalis Westminster N.V, and as such also headed two PESP studies (PESP 02146; Infrastructure Master plan Bata Port, Equatorial Guinea and PESP 03031; Offshore sand for the Construction Industry, Sri Lanka). He has well established connections with Paraguay and its authorities, as he headed 6 missions for the European Commission between 1994 and 1997 to the Pilcomayo basin, comparted by Bolivia, Argentina and Paraguay, and made 14 many visits to Asunción to help the authorities in preparing bankable reports for large infrastructural projects.

Aquatic Ecology & Water Quality Management Group

Mission

Our mission is to generate novel insights that can help to develop effective strategies for preserving and restoring aquatic ecosystems and water quality. We believe that effective management approaches require an insight in the functioning of the ecosystem as a whole. This requires linking the disciplines of ecology, eco-toxicology, environmental chemistry and mathematical modelling, and working on scales varying from microcosms

Organisation

The Aquatic Ecology and Water Quality Management group (AEW) is part of the Centre for Water and Climate of Wageningen UR (www.aew.wur.nl).

Head of the group:

Prof. Dr M. Scheffer

Additional chairs:

Prof. Dr. A.A. Koelmans: Water and Sediment Quality

Prof. Dr. J. Leentvaar: Water Management

Prof. Dr. W. van Vierssen: Aquatic Ecology

Tenured Staff:

Ir. J.J.M. de Klein: Surface water quality, nutrients, models, integrated water quality management

Dr. J. Harmsen: Sediment remediation, water and sediment quality

Dr. Ir. M. Lurling: Plankton ecology, harmful cyanobacterial blooms, trophic interactions, info-chemicals and info-disruptors

Dr. Ir. E.T.H.M. Peeters: Ecological water quality assessment, ecotoxicology, multivariate statistics, sediment ecology

Dr. R.M.M. Roijackers: Aquatic ecology

Dr. Ir. P.J. van den Brink: Ecotoxicology, community ecology, pesticides, tropics, stress ecology

Dr. Ir. E.H. van Nes: Aquatic ecology, mathematical and individual-based models

Non-tenured staff:

Ir. S. Kosten: Aquatic ecology, South American shallow lakes

Drs. J.J.C. Netten: Aquatic ecology, macrophytes

Drs. A. Poot: Sediment quality, micro-pollutants

Msc. C. Kruk : Phytoplankton ecology, South American shallow lakes

Msc. G. Lacerot: Aquatic ecology, South American shallow lakes

Drs. A. Veraart: Aquatic microbiology, denitrification

Drs. J. Zuidam: Aquatic ecology of ditches

Msc Goncalves Souza: Cyanobacteria, plankton ecology

Msc M. Rubach: Ecotoxicology

The AEW-group is a strong interdisciplinary team with great scientific recognition as well as applied impact over the past years. Our broad spectrum of expertise, the powerful ecosystem approach and the charisma of the work has started to attract top-students and 15 top visiting scientists internationally. The group has a vital network of cooperation both within and outside the team.

2.2

THE LOCAL COUNTERPARTS

1: **Ministerio de Obras Publicas y Comunicaciones (M.O.P.C.) (**www.mopc.gov.py**)**

Role in the PESP study:
• Principle counterpart for the PESP study and future client of WiseUse International and Handels & Adviesburo Visser, as envisaged investor and beneficiary of the clean-up of the Ypacarai Lake in Paraguay.
• Principal participant and decision maker in the Project Board
2: **Secretaria de Ambiente (straight under the Presidency of Paraguay).**
• Counterpart for the PESP study for environmental issues; decision maker for all actions to be taken in relation to the feasibility of the cleanup proposals.

3: **The Ministry of Industry and Commerce**
• Counterpart for all economic and import matters. Responsible for funding after a positive feasibility study
The “Ministerio de Obras Publicas y Comunicaciones” (M.O.P.C.) has been appointed by the Paraguayan Government to act as the leading ministry for the present project regarding the cleanup of the Ypacarai Lake. It is this ministry that has a long record in organizing and executing larger infrastructural projects (see: www.mopc.gov.py). Its Vice Minister, Ing. Hildegardo Gonzalez Irala (now replaced after the last presidential elections) is personally involved in all decisions and is the most important decision maker related to the project. The Vice-Minister has expressed a mayor interest in the project. For the present project the MOPC is assisted by the “Secretaria de Ambiente’ (SEAM)”, or Ministry of Environment (www.seam.gov.py).
This ministry is directly under the Presidency of Paraguay, and within its short existence (7 years now) has prepared the legislation of several laws and decrees for the protection of the biodiversity in Paraguay. To facilitate future exports to Paraguay the Ministry of Industry and Commerce (REDIEX, www.rediex.gov.py) is fully involved (and has even contributed financially, through their contract with UNDP, to the first pilot study with the biocatalyst). The responsibility of the project lays, as stated, for 100% in the hands of the M.O.P.C., while all other mentioned ministries cooperate, according to their legal status. 16

3 PROBLEM ANALYSIS
Wise Use International bv was contacted in January 2007 by the Ministry of Industry and Commerce (REDIEX) to be informed about the possibilities to clean biologically the Ypacarai Lake (Lago Azul, the blue lake as called by the Paraguayan people, most probably by the reflection of the blue skies) with its bio-catalyst “PoCo”. The Ypacarai Lake is an unique lake in Paraguay of size and importance in ecological, tourist, and hydrological sense. It has been higly polluted throughout the last decades, merely by the uncontrolled input of industrial and urban waste water. Most of the waste water of the capital Asunción are lead into the river Paraguay. However, through the urbanization, i.e. the increase of the suburbs of the capital, the waste water is now also entering the Ypacarai hydrological basin and as such resulted in an enormous increase in pollution of the lake and its surrounding wetlands.

Yet for more than 10 years the Government of Paraguay has tried to find a cost-effective solution to the pollution problem of the Ypacarai Lake. In cooperation with the Japan Agency for Development Cooperation (J.I.C.A.) several possible solutions have been presented, but never cost-effective. In view of the relatively large size of the lake, but also the shallow depth (max. 3 to 4 m in the middle part by continuous sedimentation) the s.c.

environmental dredging may not be regarded as cost-effective as the introduction of specially designed dredging equipment involves quite high costs. The latter has been confirmed by one on the leading (Dutch) dredging companies who performed a special study in 2001 to the lake.

In principle two simultaneous problems exist:

1. Wastewater treatment before entering the lake;
2. The cleanup of the lake water body itself.

As the degree of refreshing of the lake water (estimated at more than 3 years) is quite low (by the relative low hydraulic gradient of the lake basin) it will take long time before the effect of a clean water inflow restores the natural biological balance (estimated to be more than. This effect will also been retarded by the existing (partly anaerobic) lake bottom black sludgy, sometimes sticky, sediments.

Ad 1.

The Government of Paraguay has entered an agreement (of understanding) with the “Venetian low-land authorities” although the University of Padua to perform a study to map all types and magnitudes of polluters of the Ypacarai lake basin. This should lead to the design of a s.c. marginal collector scheme which leads to a series of wastewater plants. The above mentioned authority through its Italian Government has already expressed its willingness to cooperate financially.

Ad 2.

Simultaneous but separate to item 1.) it has been agreed that the only solution, regarded as cost-effective, will be the proposal by Wise Use Int. bv to restore the biological unbalance (pollution) in the lake by means of the bio-catalyst PoCo and the specially for this project designed equipment of Handels & Adviesburo Visser.

Figure 3-1 Location Ypacarai lake Paraguay

(urbanization areas marked in yellow)

3.1 LITERATURE REVIEW

1: Stormwater pollution control ponds and wetlands July 1998 by Ian Lawrence & Peter Breen 1

Table 3-1 Classes of urban pollutants and their potential impacts on receiving waters Impact on environmental values Pollutant Nuisance plant growth (including algae) Nutrients, particularly phosphorus and nitrogen Oxygen depletion of waters Hydrocarbons (chemical oxygen demand) Organic materials (biological oxygen demand) Light modifying substances Suspended solids (soil particles, organic material) Toxicants impacting on the physiology of plants and animals Heavy metals (chromium, copper, lead, zinc), pesticides Pathogens, potentially impacting on human health Faecal bacteria, viruses.

The sedimentation of organic material discharged by a storm /heavy rainfall event leads to rapid growth of the benthic microbes that normally feed on decomposing organic material at the bottom of the pond or wetland. Their growth depletes oxygen in the water column and sediments, with the potential to create anaerobic conditions. If organic material remains after the oxygen has all been used up, further microbial growth leads to the transformation of a number of pollutants (such as nitrate, ferric iron, sulphate) and their release (as ammonium ions, nitrogen, phosphate, hydrogen sulphide) back into the water column in soluble or gaseous forms. Soluble forms are highly available for uptake by biota; gaseous forms are lost to the atmosphere.

1 Stormwater pollution control ponds and wetlands July 1998 by Ian Lawrence & Peter Breen. Cooperative research centre for fresh water ecology, Australia. ISBN 1-876144 20 318

This release can significantly offset the interception performance of the pond or wetland. It is more likely to occur in ponds that are too small for their purpose or in which the BOD load is not well distributed across the pond area, or in ponds that can suffer stratification. Stratification enhances the likelihood that reducing conditions will occur. Research indicates that turbid pools, ponds and lakes are highly susceptible to thermal stratification under typical summer conditions throughout temperate areas. Stratification (formation of layers of different density and/or temperature) blocks the transfer of oxygen from the water surface to the sediments to replace the oxygen used up by BOD during the decomposition of organic material. However in (sub)tropical conditions this stratification is less observed.

Wetland water quality and ecology

Many of the changes observed in sediment processes during wetting and drying cycles occur as a result of the significant differences between aerobic and anaerobic decomposition processes. Decomposition in aerobic conditions or during drying phases occurs through the action of a range of organisms including invertebrates, fungi and bacteria. Aerobic decomposition is an efficient process resulting in near complete and rapid degradation of organic material, high energy yields and high assimilation rates. Decomposition in anaerobic conditions during the wet phase occurs almost entirely through the action of anaerobic bacteria. Fermentation is the major anaerobic decomposition pathway and it results in slow, incomplete degradation of organic matter, low energy yields and low assimilation rates.

The main end products of organic decomposition in well drained soil are carbon dioxide gas (CO_2), nitrate (NO_3^-), sulphate (SO_4), and a small amount of resistant residues (soil humus). In submerged soils the end products are CO_2, hydrogen gas (H_2), methane gas (CH_4), ammonium ion (NH_4^-), hydrogen sulphide gas (H_2S), amines, mercaptans and a large proportion of partially humidified residues (peat) (Ponnamperuma 1972, 1984). The organic or peat soils of many permanently inundated wetlands are evidence of the incomplete decomposition process.

In-wetland interception of pollutants

Perennial wetlands intercept dissolved and colloidal forms of nutrients associated with attenuated flow or base flow. The benthic biofilm adsorbs colloidal nutrients and transfers them to the sediments, while dissolved nutrients are primarily taken up by the benthic and epiphytic algae. Adhesion of fine particles onto vegetative surfaces may also play an important role in wetland interception. The macrophyte substrate and biofilm treatment zones and the macrophyte humus accumulation— adsorption treatment zones are essential for successful treatment of these forms of pollutants.

Ephemeral wetlands When storm runoff is infrequent, or in areas naturally subject to periodic flooding, ephemeral wetlands may be appropriate to control pollutants. In ephemeral wetlands the pollutants are intercepted mainly by adhesion to vegetative surfaces and sedimentation (enhanced by evaporation, evapotranspiration and leakage of the ponded water into the groundwater). As in other wetlands, microbial and vegetative processes incorporate sedimented materials into the soil forming the bed of the wetland. As the ephemeral wetlands and their sediments dry out, the sediments' aeration (oxygen supply) improves, increasing the rate of decomposition of organic material and assisting the long-term management of nutrients The wetting and drying cycle is central to the sedimentary processes in ephemeral wetlands.

Flooding reduces the oxygen diffusion rate in the sediments by a factor of 104, altering a number of redox reactions and changing the pathways of biological metabolism from aerobic to anaerobic.

The breakdown of molecules by hydrolytic enzymes is the critical process that determines the rate of decomposition of organic materials: in this process, large organic molecules 19 disintegrate to a size which bacteria are capable of assimilating (Lock 1994).

Macrophytes continually supply organic material to the biofilm in their root zone. This supply maintains the concentrations of enzymes that hydrolyse polymeric material in the near-plant biofilm. In bare sediment areas (the bare lake bottom like in the Yapacarai Lake, note: WiseUse), the enzyme concentrations in biofilm are much lower.

Macrophytes are important also because they transfer oxygen to the sediment via their rhizomes.

There are few reports of the rates at which carbon or nutrients are taken up by biofilm in freshwater ponds, wetlands and lakes. Take-up rates for dissolved organic material are reported as ranging from 3 to 1000 mg/m2/hr for river epipelon layers. In wastewater biofilm systems (trickling filters, maturation ponds), on the other hand, take-up rates range from 60 to 400 mg/m2/hr Since ephemeral wetlands have a long retention time, researchers assume that most of the pollutants captured from an event are retained within the ephemeral wetland system.

Some remarks on the Water Hyacinth: (Camalotes in South America) These are also present at the inlets and outlets of the Ypacarai Lake. The water hyacinth spreads in water environments such as bays and inlets with the following conditions: quiescent water; shallow depths (< 6m); bed surface covered with deposited sediments rich in organic matter and availability of key mineral elements namely nitrogen and phosphorus in the nutrients. *Chemical composition Water hyacinth comprises 95 per cent water and only 5 per cent dry matter of which 50 per cent is silica, 30 percent potassium, 15 per cent nitrogen and 5 per cent protein*.

From the unique chemical content of the water hyacinth, its beneficial uses are limited.
The water hyacinth:
- cannot be used as a livestock feed because it contains too much silica, calcium oxalate, potassium and too little protein.
- cannot be directly used as a fertilizer because its C:N ratio is too high necessitating addition of N-fertilizer.
- cannot be used poor raw material for paper, mats or ceiling boards because its fibre length is too short, A few beneficial uses have been identified but the
- Large scale production is uneconomical when compared with the negative effects attributed to the water hyacinth field. Such beneficial uses include biogas production and removal of heavy metals from industrial pollution when water passes and is sieved by the water hyacinth fabric.

Under favourable conditions the population of water hyacinth doubles between 5 – 15 days.
If completely undisturbed its biomass weighs 25kg per square metre or 400 tonnes per hectare. Each flower produces a seed pod which can contain upto 200 seeds. The seed can remain viable for upto 15 years in water, silt or mud. The plant can also propagate vegetatively by sending out runners into the water which produces daughter plants that can reproduce at about the 2-week stage.

Removing aquatic plants but no sediments:
The aquatic plant community may reduce the concentration of nutrients in the water column but it did not significantly affect the sediment concentrations. Harvesting the hydrophytes removed the equivalent of 58–88% and 39–78% of the nitrogen (N) and phosphorus (P) load associated with the water column, respectively. In contrast, the harvests accounted for only 1–2% of the N and P load associated with the sediments Some notes on macroinvertebrates (in the biofilm of wedlands and lakebottom).

Historically, their abundance and diversity have been used as an indicator of ecosystem health and of local biodiversity. They are a key component of the food chain. Most indices that are used to determine water quality rank the various forms of benthic 20 macroinvertebrates with respect to pollution sensitivity. The presence of pollution sensitive macroinvertebrates indicates that the body of water is healthy.

Alternatively, the excessive
presence of pollution tolerant macroinvertebrates indicates poor water quality. Ideally, a healthy body of water will hold an abundant and diverse macroinvertebrate population. The macroinvertebrates traditionally seen as being pollution sensitive include: mayflies (Ephemeroptera), caddisflies (trichoptera), and stoneflies (Plecoptera).

The macroinvertebrates that have been traditionally considered pollution tolerant include: aquatic worms (Oligocheata), leeches (Hirudinea) and non-biting midges or commonly known as "blood worms" (Chironomidae). Flow, food, habitat and water quality are the primary determinants of macroinvertebrate abundance and diversity.

Food sources
include phytoplankton, biofilms (i.e. the layers of bacteria or other micro-organisms that cover submerged surfaces) and terrestrial organic material (e.g. leaves) that enter the water from the riparian vegetation. Major predation occurs from other macroinvertebrates and fish. Key habitats for macroinvertebrates are the benthic sediments, aquatic vegetation and woody debris.

2: The restoration of the water quality of Lake Rodó, Montevideo Uruguay
2 Lake Rodó is a very small lake in Montevideo and possees a turbid system, a condition attributed to algal biomass. The proximal source of the eutrophication was stormwater discharges from an ill-defined urban area.

An attempt was made to restore the water quality of Lake Rodó, the first time this has been done in Uruguay. In spring 1996 it was drained, sediments were removed and stream inputs were diverted. Groundwater was used to re-fill the lake. Due to its high nutrient concentration a re-circulation system was designed, pumping water from associated pools covered with free-floating plants.

2. After the lake was refilled, the system was characterized by oxygen saturation or over saturation, neutral to basic pH, and high phosphorus, nitrogen and silicate concentrations. Ratios of total nitrogen (TN):total phosphorus (TP) and chlorophyll a (Chl a):TP indicated that phosphorus was the primary limiting nutrient during the period of groundwater supply. Once groundwater pumping had ceased, there was a decrease in TN:TP and Chl a:TP ratios, suggesting N-limiting conditions prevailed in some periods.

3. Before restoration, the phytoplankton community was dominated year-round by *Planktothrix agardhii*; since restoration the community has been more diverse. This change has favoured grazing by mesozooplankton, and the onset of clear-water phases in spring.

4. Abundant populations of small omnivorous fish maintained a high predation pressure on zooplankton, restricting the abundance of large-bodied herbivores, which, in turn, allowed an increase in phytoplanton biomass and a decrease in water transparency. Based on this observation, together with the phosphorus concentration and the low abundance of filamentous cyanobacteria compared with previous studies, we suggest that top-down control has played a key role in increasing transparency in Lake Rodó.

5. A nutrient reduction programme, by the mechanical harvest of floating plants, and a removal of small omnivorous fishes and stocking strictly with piscivores, could be key factors in the achievement of a stable clear-water phase.
2 Aquatic Conservation: Marine and Freshwater Ecosystems Volume 11, Issue 1, 2001. Pages: 31-44 Limnological changes in a sub-tropical shallow hypertrophic lake during its restoration: two years of a whole-lake experiment F. Scasso *, N. Mazzeo, J. Gorga, C. Kruk, G. Lacerot, J. Clemente, D. Fabián, S. Bonilla Sección Limnología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay 21
However, if blooms of Microcystis or other similar genera occur in summer, additional measures (e.g. reduction of the hydraulic residence time) will be needed to improve water transparency.

3. The structuring role of macrophytes on trophic dynamics in shallow lakes
under a climate-warming scenario.
3 A drastic reduction of external nutrient loading seems to be the best method for restoring small or large subtropical and tropical lakes, but the scientific basis (e.g. nutrient threshold levels) to make decisions remains limited. Focus may well have to be on reducing external N loading as nitrogen has often been found to be the limiting nutrient for phytoplankton growth in tropical lakes (Lewis, 1996).

For small and large shallow lakes, the best strategy for improving the environmental state is external nutrient loading reduction.
Like small lakes, large shallow lakes also may respond strongly and quickly to loading reduction. For smaller lakes resistant to nutrient loading reduction, physico-chemical and biological methods may enhance recovery. However, these methods are unlikely to be feasible in large shallow lakes (costwise note: WiseUse).

Sediment removal and chemical treatment, in particular, are less attractive because:
a) sediment transport and storage on land (if removed) will be expensive;
b) there is a large risk of sediment redistribution from resuspension;
c) the sediment P pool (Phospate ??) often is relatively small in large lakes
Fish manipulation also may be impractical because:
a) the large size makes it difficult to obtain a strong effect on the target fish population;

b) fish population is among the fi rst to respond to nutrient loading reduction, and natural changes in fish stock become diffi cult to discern in large lakes.

Differences in biological interactions in cold temperate versus warm temperate subtropical-tropical lakes make it difficult to apply cold temperate biological restoration methods to warm lakes. Warm lakes often have prolonged growth seasons with a larger risk of long-lasting algal blooming and dense floating plant communities, higher dominance and abundance of small fish, higher fish aggregation in vegetation (loss of zooplankton refuge), more fish cohorts per year, and more omnivory by fish and less specialist piscivory.

Warm lakes differ in trophic structure depending on their location in wet or dry regions, and on temperature and precipitation changes. Both (sub)tropical and Mediterranean lakes are very sensitive to changes in waterlevel (Coops et al. 2003, Mazzeo et al. 2003). Hydrological changes can deeply affect water transparency, the development of submerged plant, the structure of the fish communities, and salinity.

These characteristics have to be seriously considered when designing restoration strategies for specific lakes.
A higher importance of nutrient loading for the functioning of warm lakes than in comparative temperate lakes has been found in field studies in Mediterranean lakes (Romo et al. 2004) and in an experimental study along a latitudinal gradient in Europe (Moss et al. 2004).
Regardless of lake size and climate region a drastic reduction of the external nutrient loading seems to be the best way forward for restoring eutrophic lakes.
However, the scientific basis on which to decide either restoration (such as the targeted
3 PhD thesis, 2006 Mariana Meerhoff Department of Freshwater Ecology:

Department of Biological Sciences,
University of Aarhus. Denmark22
nutrient and fish threshold levels), or management (targeted water level due to water extraction or irrigation) strategies, is still very limited for warm lakes.

Comments by WiseUse on some aspects mentioned in the literature Review.
The Ypacarai Wetlands are partially ephemeral (dry during dry periods) and partially perennial. This makes them of vital importance as a first bufferzone against pollution.

Ian Lawrence & Peter 1) write: As the breakdown of molecules by hydrolytic enzymes is a critical process that determines the rate of decomposition of organic materials and thus, large organic molecules disintegrate to a size which bacteria are capable of assimilating (Lock 1994). The biocatalyst PoCo's function is just to stimulate this process. The bare lakebottom of the Ypacarai lake has probably very little Macrophyte activity. Also this is important to be re-activated as microphytes are important because they transfer oxygen to the sediment via their rhizomes.

The waterhyacinth is present in the Ypacarai lake but (still) not abundant. The wind and wave action pushes it towards the outlet of the Rio Salado and the inlet of the Yuqury stream. Reducing the nutrients is therefore important.

All recommendations written in the PH.D thesis of Mrs. Mariana Meerhoff 3) imply that the only feasible solution is the reduction of the P and N load. This prevention method seems positive and necessary. However the recuperation of the lake itself without doing anything to the lakebottom is not a question of 1 or 2 years, but takes much more time.

If a biocatalyst can stimulate the (mostly anaerobic) biofilm of the lakebottom, then it is worth trying. Quite positive effects have been reported from the experiments in the semi confined pond next to the lake. The effect of introducing the biocatalyst in the lake bottom sediments could provoque the critical process of moleculair breakdow of the organic sediments of the lakebottom by hydrolysis.

Meerhoff writes: "*However, the scientific basis on which to decide either restoration (such as the targeted nutrient and fish threshold levels), or management (targeted water level due to water extraction or irrigation) strategies, is still very limited for warm lakes*."
The best solutions are not invented by waiting on sound scientific basis, but to provide their basis by experimenting (Note WiseUse: preferably on a scientific well designed basis).

3.2 PREPARATORY ACTIVITIES PERFORMED BY WISEUSE

In determining the feasibility of the developed biocatalyst in a (sub)tropical environment and more specifically in the Ypacarai Lake, a series of first pilot tests took place in March 2007 at the laboratories of the "Universidad Nacional de Asunción", faculty of biochemistry (CEMIT). All tests have been performed by a Wise Use biochemist in cooperation with the University personnel, with presence of several engineers of the cooperating ministries.
The purpose of this pilot study was to confirm the efficacy of PoCo i.e. bio-catalyst to decrease the pollutant levels in the Ypacarai Lake which will help to clean up the Lake.

3.2.1 THE OBJECTIVE OF THE LABORATORY TESTS CARRIED OUT IN THE PILOT STAGE

As every lake or river contains its own biodiversity and chemical compounds (constituents) it is every time necessary to prove the catalysing effect of PoCo in the target area; both in an aerobic and in an anaerobic environment. More precisely: to determine if a positive effect could be proven, where important micro organisms could be reactivated, multiply, and further help to restore the ecological balance and to improve 23 water quality in a short specific time period. This results then in safe lake water for both intake and recreation.
The efficacy of PoCo was proved in the pilot studies to some extent, with a reduction in pathogenic micro-organisms (fecal coliforms and salmonella) and increase in dissolved oxygen levels to the maximum possible level thereby restoring the flora and fauna of the ecosystem.
The report of the pre-PESP Pilot studies and its conclusions is annexed to this report. The pilot test series were financed by Wise Use and through the Ministry of Industry and Commerce (REDIEX) through UNDP funding.

3.2.2 MASTER PLAN OF ACTIVITIES

The biochemical cleanup of the Ypacarai Lake is proposed on the basis of a master plan, and contains the following activities:

• Within a 5 years period all sewer lines within the hydrological Yapacarai basin will be diverted to marginal collectors and to water treatment plant(s). These projects / works will be designed, executed and funded by Paraguay itself.

• The 'Secretaria de Ambiente'(SEAM) already mapped around 150 small and medium large polluting companies. Some of them, identified as mayor polluters, have already been ordered to install (or repair their existing) treatment installations. The major polluters are slaughterhouses, leather-, oil- and food processing, paper, and few small scale metallurgical industries.

• The present water treatment plants for drinking water in San Bernardino and San Lorenzo have to be upgraded according to modern standards.

• In 2003 the organization "Fundapueblos" has introduced a special programme to promote the environmental importance to all mayors and councillors of the surrounding villages. This programme continues during the cleanup, with emphasis on prevention and public environmental awareness.

• In cooperation with the University of Padua (Italy) and the s.c. Authority of the Venezia Basin a masterplan has been proposed to clean the surrounding wetlands via "phyto-remediation" reinstating their buffering capabilities to pollutants. Part of this is closely related to the installation of marginal collector network.

• The cleanup of the Ypacarai Lake itself by the application of the bio-catalyst of Wise Use and using the special designed hardware of Handels & Adviesburo Visser. The complete engineering and application will be done by the Dutch Consortium and will be proposed to the client on a turn-key basis.

3.2.3 HIGH LEVEL DESCRIPTION OF ACTIVITIES PARTIES INVOLVED

AEW (Wageningen) activities in the PESP-proposal Lago Ypacarai

Introduction

Lago Ypacarai suffers from ongoing pollution through discharge of untreated urban waste water. The urban and industrial effluents can contain high concentrations of nutrients, large amounts of sediment, oxygen-consuming wastes, pathogens and a whole palate of toxic substances (heavy metals, oil and pesticides). The high nutrient loads promote 24 harmful cyanobacterial blooms and the accumulated organic matter might cause severe oxygen depletion. The variety of water quality problems can be harmful to humans and cattle, limit recreational activities, and decrease fisheries.

WiseUse International BV (The Netherlands) has proposed a restoration of the Lago Ypacarai through application of the biocatalyst “PoCo”. However, management and restoration of the damaged aquatic system effectively, requires more research and knowledge than has been gathered up to now. The AEW group will contribute to the scientific research through cooperation with scientists of the University of Asuncion, through training/coaching of PhD-students, and advising/consultancy by in-house experts.

WiseUse International and Handels & Adviesburo Visser:
Application and quantity control of PoCo within the test area of the Ypacarai Lake:
The application of the product PoCo is already explained in chapter 3.1.1 PoCo acts as an Organic Stimulators which accelerates/boosts the growth of micro-organisms in a natural way by supplementing micro-nutrients and trace elements which helps to enhances the rate of Microbial and Bio-Chemical Reactions. In other words PoCo promotes maximum possible degradation of complex organic compounds to simpler ones resulting in a well balanced and natural microbiological environment. However this application of the Bio catalyst should be monitored carefully and scientifically guided. This is why the below stated cooperation with the University of Wageningen and the University of Asunción is of the greatest importance.

3.3
RESEARCH

The tests carried out in the laboratory of CEMIT with the product “PoCo” did and could not fully meet the required scientifically standards since it only was carried out to get the preliminary information of how the project should be executed; i.e. to design the plan of action to clean up the Ypacarai Lake. Controlled in-situ experiments are essential and should at least test the effectiveness and possible side-effects of the product. Moreover, the objective is restoration of the damaged lake, which requires a comprehensive problem description of the problem, studies on several potential restoration measures, including controlled experiments at different scales, and above all elimination of incoming pollutants.

1) Description of the in-lake situation: A first impression will be obtained by Ir. Kosten during her first visit to the lake. Keywords are: Nutrient balance, hydrology, sediment thickness and composition (including micro-pollutants and heavy metals), 25 phytoplankton composition with emphasis on toxic cyanobacteria, and standard limnological data.

2) Experiments with PoCo: So far, Poco has been tested on sludge reduction in large tanks. It remains to be tested on a real scale whether PoCo improves the overall water quality. Release of nutrients or other bio available compounds from the sediment might have an effect on water quality. In this the high phosphate concentrations found in the above mentioned large tank study need to be taken seriously as they might promote harmful cyanobacterial blooms. Investigators should especially focus on the release of nutrients and pollutants and test the product in combination with other products (such as phosphate fixators).

3) Experiments with alternative measures: Inasmuch as the success of Poco can not be guaranteed *a priori*, and perhaps other measures or treatments are needed separately or in combination with Poco, the scientists ould include alternatives (such as dredging) in their design, which also includes the possibility of treating only parts (recreational area) of the lake.

4) Beside the experiments in laboratories, trusted and tangible results can be obtained performing in situ, thus in the lake it self. Criticasters complain about the small scale of the laboratory tests. They do not represent a real situation because there is not a complete equal environmental situation in the lab that simulates the lake’s. Therefore Wise Use and CEMIT agreed upon the execution of a real scale test in the swimming pools of the Nautic Club, which is directly connected to the lake. Besides the effect on growth of algae’s and coliforms, the sediment and the physico-chemical parameters of the treated water will be analysed.

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3.4 THE PROJECT'S OBJECTIVES AND DELIVERABLES

Table 3-2 Project objectives and deliverables

3.4 THE PROJECT'S OBJECTIVES AND DELIVERABLES

Table 3-2 Project objectives and deliverables

Objectives	Description
Technical objectives	Assessment of the in situ status of the pollution, definition of all important items and parameters, and the assessment of the applicability of bio-catalyst for the biochemical cleanup of the Ypacarai lake and all its technical implications and requirements. The analysis of the pollution, the tests carried out and gathering of all information required to translate this into technical equipment required (Capex) and its running costs.
Organisational & institutional objectives	Definition of the project and its stakeholders, e.g. the various ministries, NGO's, research institutes etc. Its decision making structure will need to be defined. Cooperation of universities and student interchange
Financial objectives	The successful application of a general treatment scheme inclusive the applicability of bio-catalysts in this heavily polluted lake, its sub-bottom and influents, in order to reduce the necessity for (partly) expensive dredging.(could be a reduction in costs of more than 70 %).
Economic objectives	Cost-benefit analysis of the project, calculating the projects IRR and NVP.
Legal objectives	Preparation of preliminary contracts withy the Government of Paraguay for equipment supply and bio-catalysts. Preliminary contracts signed by the client for equipment supply and bio-catalysts.
Environmental objectives	To restore the natural biological balance in the lake and the bordering wetlands, creating a healthy environment for all players in the Ypacarai basin.
Marketing objectives	Preparation of commercial proposals detailing all equipment required, its Capex and Opex. Proposals and commercial offers detailing all equipment needed and bio-catalysts, both from Wise use and Visser Handelsburo.

4 TECHNICAL OBJECTIVES

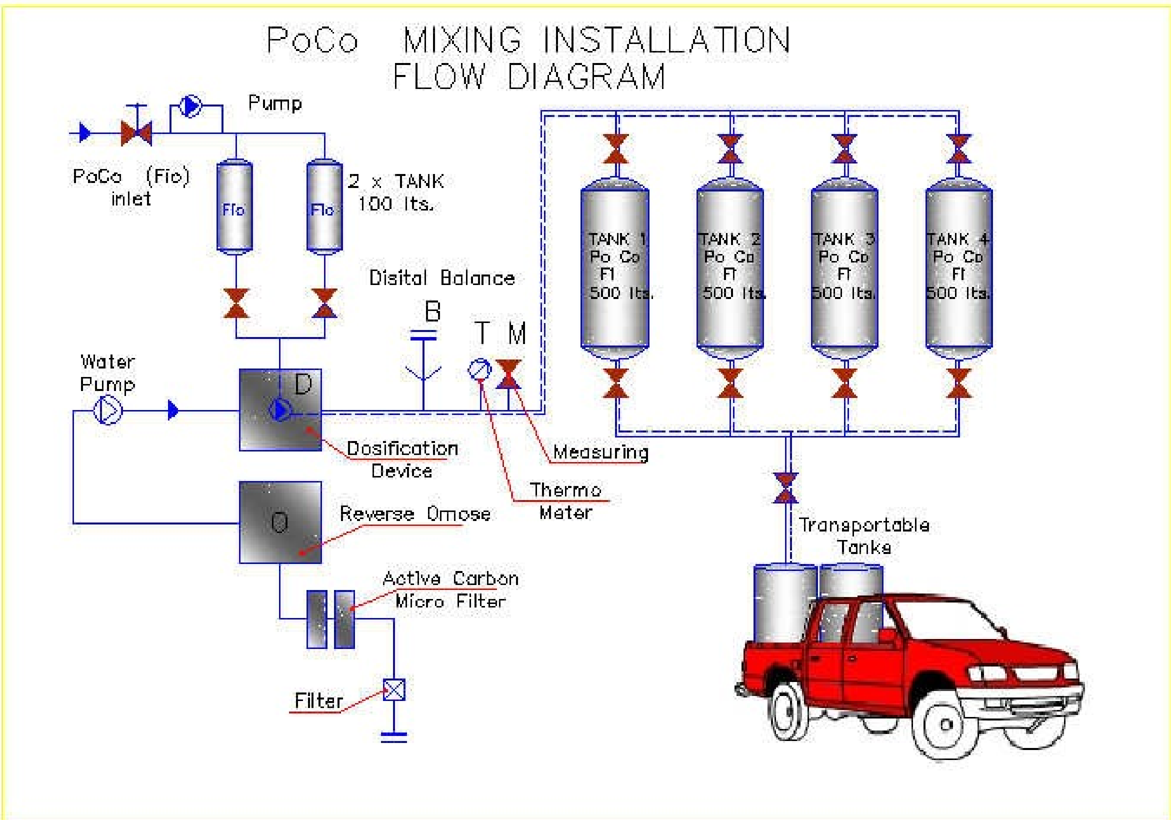
4.1 ACTIVITIES CARRIED OUT IN THE NETHERLANDS

4.1.1 ASSESSMENT IN SITU APPLICABILITY BIO-CATALYST

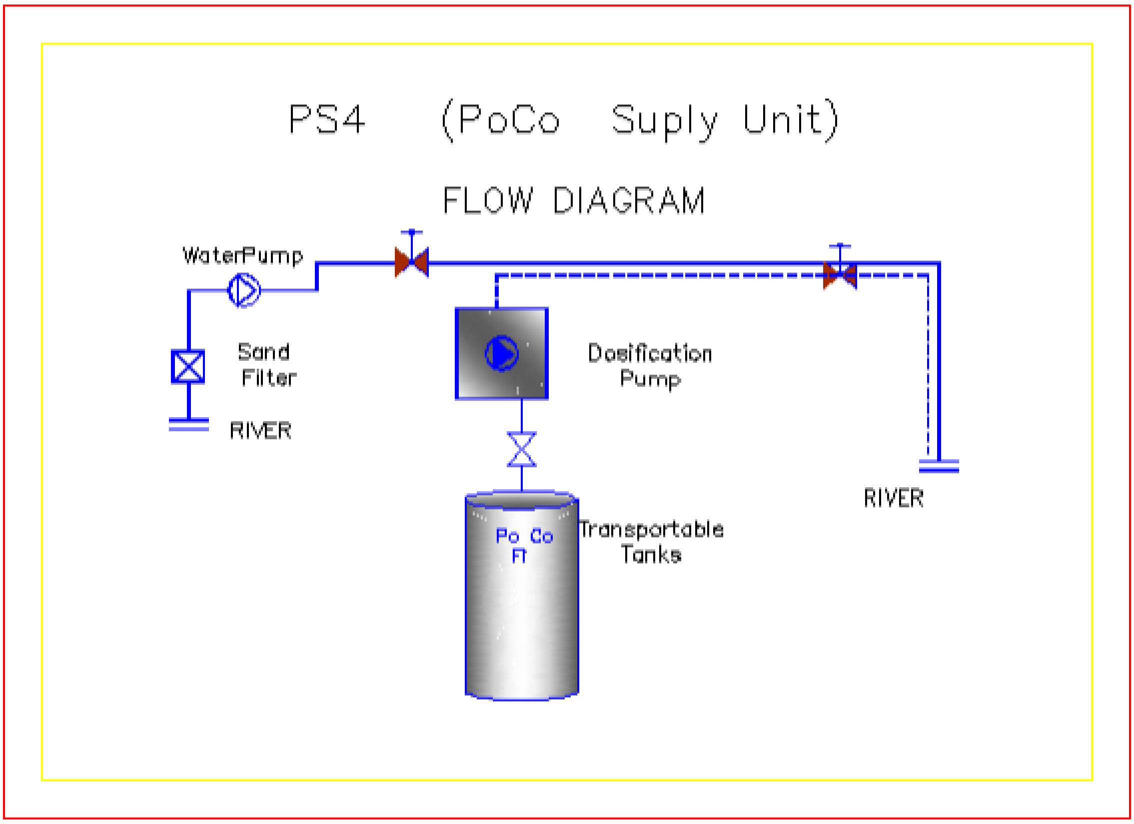
In February 2007, Wise Use has conducted its first laboratory tests in cooperation with CEMIT targeting the nature and extent of the lake pollution. The chief pollution stems from the untreated pollution in the lake. The water is polluted with high concentrations of colform bacteria's and blue algae that produce cyano bacteria's.
The POCO product that will be used therefore differs from the standard product as these high concentrations are not common in the Netherlands. The bespoke Poco product was sent to Paraguay. WUR also received one liter of this special product.This adapted version of Poco contains additional components that ensure a quick reaction in the pool water.

4.1.2 ENGINEERING MIXING STATION, POCO SUPPLY UNITS AND SELF PROPELLED PONTOONS

PoCo will be delivered on site in a concentrated liquid (F-10 concentration) along with small mixing installation, this will help to prepare the ready to use end product at site.
A small mixing installation will be supplied. The product PoCo will be delivered on site in a concentrated liquid (F-10 concentration). This means that on site some processing has to take place to produce the ready end product.
The mixing plant consists of storage tanks for half-fabricates, a mixing pump, a small treatment installation on the basis of reverse osmosis with UV-sterilization and storage tanks for the end product, ready to be used.

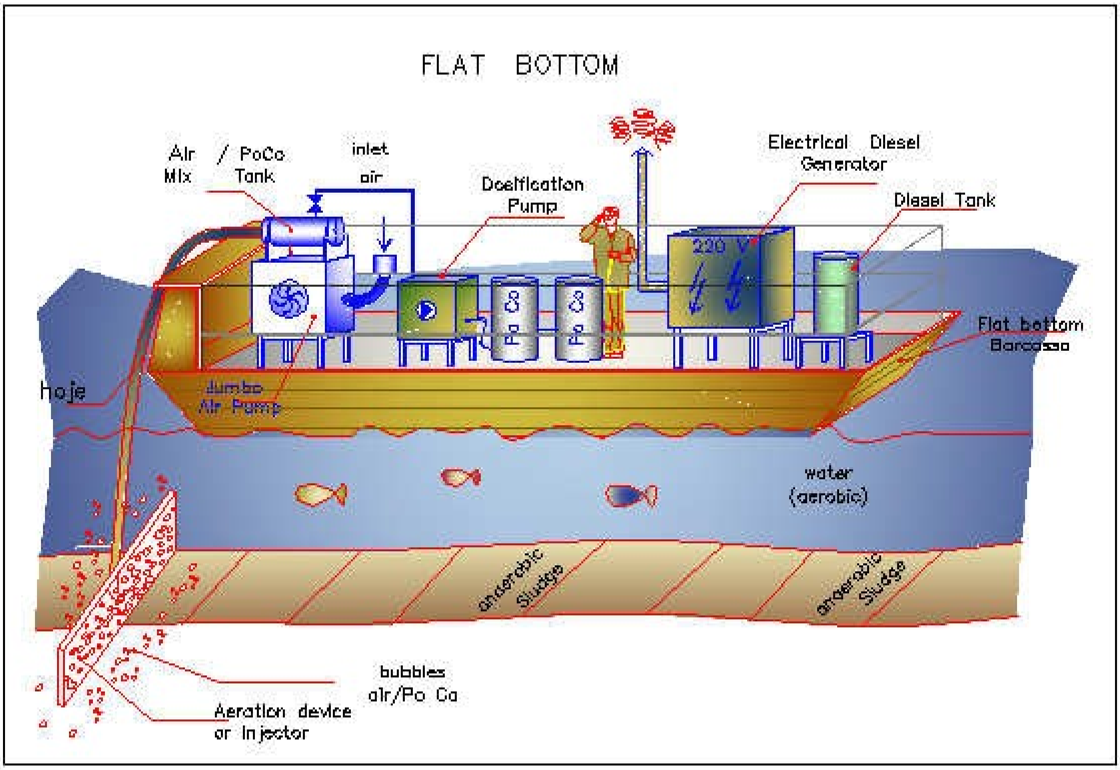


P.S.U. (PoCo Supply Unit)
The Poco supply unit contains various components, a.o. a dosing pump, a transportable stainless stell tack with valve and required instrumentation for measurements of flow, time and level of the tank. The dosing pump uses low level of energy which can be generated with a sun panel producing 12 Volt DC.



Self propelled pontoons.
As it never can be the intention to introduce PoCo in the future in an uncontrolled way into the lake, it is therefore of the greatest importance to quantify the dose and to determine the exact locations and depths of the products introduction.
Not every random location is suitable. To establish the quantities of the product and the locations a good knowledge of the lake geometry and its internal water currents is obligatory. Hydrological sources indicate that the lake water refreshment may take about 3 to 5 years (depending of the annual precipitation). As stated above, the polluted bottom sediments also have to be taken into account, implying an introduction of the product PoCo in both the lake water and into the mostly sludgy bottom sediments.
To realize this, special designed “flatbottoms or self propelled pontoons” will be used, each equipped with an electric aggregate for “aeration plates” and dose units. It is estimated that 2 flatbottoms are necessary, each of them navigating according to a fixed pattern across the lake to inject PoCo into the (mostly) anaerobic sludgy bottom sediments. The flatbottoms will be provided locally but the apparatus will be designed and delivered by Handels & Advies buro Visser.

As stated, existing water currents will be taken into account. All three flatbottoms will cover an area and introduce the product. Mostly semi-circular manoeuvring will be done, from outside to inside. The highest concentration of polluted sludge is in the middle and western side of the lake, as an effect of wind and currents. Water depths are between 1 and 3 to 4 meters and the average thickness of the polluted anaerobic sludge is estimated to range from 0.2 to 1.5 m.



If the injection into the sludgy bottom sediments is difficult to realize, alternatively the end product Poco (on F1-concentration) may be mixed with sand and gelatine to produce a more heavy and sinkable form of the PoCo containing product. This will sink into the bottom sediments and as the gelatine slowly dissolves the contaminated water and sediments will be activated by the bio-catalyst.

It is also foreseen to introduce PoCo by a specially designed PSU (PoCo Supply Unit in the (still polluted) upstream part of the inlets (streams) of the lake. This activates mainly the aerobic part of the waste water before entering the lake.

An important factor in reducing pollution is awakening /awareness of the population and the factories of the need to handle waste products in a right way. But this awareness will be more effective if one sees the benefits of a healthy environment, with all adjoined economic benefits. The Fundapueblos foundation is already quite integrated in local primary and secondary schools by supporting any initiative serving this purpose.

4.1.3 EXPORTING CONCENTRATES & ADDITIVES IN ORDER TO PRODUCE POCO

PoCo in concentrated form was sent to Paraguay to conduct the test in swimming pool of the club Nautico. The product had been adapted catering the special needs and requirements given the high pollution levels and the biochemical composition of the water and sediment. Moreover, it was condensed to ease the international transport, Also, separately, additions and reaction enhancers were added in Paraguay.

4.1.4 MANUFACTURING F1 DOSES

PoCo was sent to Paraguay was condensed to a factor 10, being, being the so-called F1 doses. In Paraguay purified water was added transforming it into F10 (9 parts water, 1 part F1). In total 18 liters F1 was sent to Paraguay, a sufficient quantity for the test and various replications. 30

4.1.5 SECOND TESTS

After studying the results of the Pilot test in March 2007, it appeared that further study was needed. Despite the promising results, the set up was at a too low scale (3*500 litre tanks) to draw definitive and incisive conclusions. It became clear that “something” happened, but the exact nature of this phenomenon could not be defined due to small volume. Following advice of CEMIT it was decided to start a large scale test in the lake and further laboratory investigations.

1. Lake tests. It was firstly intended to create two small artificial lakes at the discharge of the River Yuguyry on a government owned piece of land. It was intended that the MOPC would dig these holes. The intention was that the Ypacarai aquatic situation could be mimicked. Eventually, based on costs considerations it was decided to undertake the tests in the Club Nautica swimming pools. The water in these pools are in direct contact with the lake and have the same biochemical content and sedimentation. The MOPC asked for permission and given that it was outside the tourist season, this was granted.

2. Further laboratory investigation: WUR and CEMIT have given their own methodology to further investigate PoCo on a laboratory scale. WUR predominantly aims on investigating the effects of Poco on decreasing sedimentation. CEMIT aims on studying the Poco effect on elimination of coliform bacteria’s and algae. Both research institutes were responsible for their own protocols and should report to Wise Use their results and analysis. CEMIT did, WUR (AEW). The WUR (AEW) did want a much more extended test series and demanded to have many more variables at their disposal before they really wanted to cooperate with any in situ testing. Yet, as this both cost wise and within the available timeframe not could be realized Wise Use felt their hand rather forced and decided to continue according to CEMIT’s procedures.

The AEW comment by letter to the EVD is depicted below.

Onderzoek naar de werkzaamheid van PoCo
Voortgangsrapportage aan EVD dd 23 april 2008

Introductie
PoCo wordt verkocht als een biokatalysator die de afbraak van organisch materiaal stimuleert, de helderheid van water kan verbeteren en de groei van cyanobacteriën kan remmen. Er wordt overwogen het product in te zetten om de waterkwaliteit van het meer Ypacaraí aanzienlijk te verbeteren. Het meer is erg saproob (bevat grote concentraties organische stoffen) en er treden regelmatig blooms van cyanobacteriën op.

Hypothesen:
1. PoCo stimuleert de afbraak van organisch materiaal
2. PoCo vermindert cyanobacteriën groei
   a. Het verwijdert cyanobacteriën blooms
   b. Het voorkomt de vorming van cyanobacteriën blooms
3. PoCo stimuleert de mineralisatie van organisch materiaal en vermindert gelijktijdig cyanobacteriën groei.

Test 1:
Verschillende concentraties PoCo (10ml PoCo op 1 liter water tot 10^{-4}ml PoCo op 1 liter water) en een referentie (geen PoCo toegevoegd) worden in drievoud geïncubeerd met water uit Lago Ypacaraí bij constante temperatuur van 25^0C (zie foto). Vooraf en 2,5 week na toediening zal de concentratie organische stof worden bepaald.
Test 2:
Idem aan 1, maar nu niet met water uit Lago Ypacaraí, maar met de dominante cyanobacteriënsoort uit Lago Ypacaraí (Microcystis aeruginosa). Op verschillende tijdstippen zal de concentratie van deze cyanobacteriënsoort worden gemeten.
Test 3:
Indien uit test 1 en 2 blijkt dat PoCo inderdaad de afbraak van organisch materiaal stimuleert en de groei van cyanobacteriën remt, dan wordt een combinatie van test 1 en test 2 ingezet. Dit is nodig omdat bij de afbraak van organisch materiaal voedingsstoffen vrij komen die de cyanobacteriëngroei kan stimuleren. Het is dus van belang om te weten of cyanobacteriëngroei ook geremd wordt bij de toevoer van deze extra voedingsstoffen.

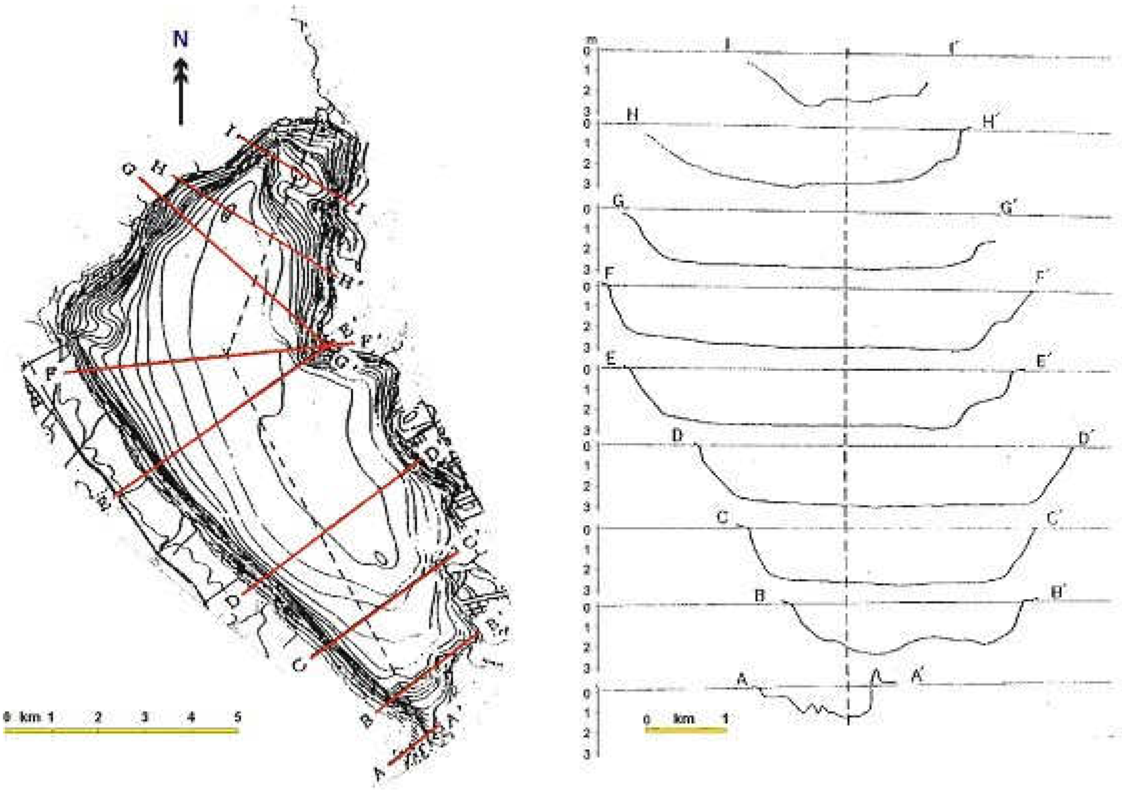
Tijdsplanning:
Op 28/3/2008 hebben we een fles PoCo mogen ontvangen. We verwachten 2 á 3 maanden nodig te hebben om conclusies te kunnen trekken over de effectiviteit van PoCo.

Disclaimer
Wise Use heeft op eigen initiatief ook een proef ingezet in een afgesloten gedeelte van Lago Ypacaraí. Wij hebben deze proefopzet vooraf bekritiseerd vanwege het ontbreken van referenties en replica's. We vinden de proefopzet wetenschappelijk ontoereikend en zullen dan ook geen wetenschappelijke uitspraken doen over de werkzaamheid van PoCo op basis van de resultaten van deze proef.

Miquel Lurling
Sarian Kosten

4.1.6 PREPARATION OF EQUIPMENT FOR BATHYMETRIC ANALYSES (BY SOUNDING

In 1990 a bathymetric analyse was carried out in the lake. The idea was also to include a similar analysis in order to obtain specific information about the sedimentation in the lake. Comparing the current with the older results could lead to more detailed information about the increase or reduction of sediment. Due to technical and financial reasons these test were not executed during this study and postponed to another moment, still to be defined.



Carta batimetrica e profilo del lago (1990)

4.1.7 PREPARATION FOR INSTALLING PUMP INSTALLATION.

Handels & Advies buro Visser has calculated required capacity (M3/hour) of the pumps. Given that about 6000 M3 content of two centrifugal pumps it is required that sufficient capacity to create a modest flow in the swimming pool similar to the natural flow in the lake. The flow ensures the dispersion of PoCo in all parts of the swimming pool. Further pipes and connection material were calculated too after the drawing (on scale) were send to the Netherlands.

4.2 ACTIVITIES CARRIED OUT IN PARAGUAY

4.2.1 ASSESSMENT IN SITU STATUS OF THE POLLUTION AND PARAMETERS DEFINITIONS

THE HYDROLOGIC BALANCE OF THE LAKE

In a combined study the JICA (Japan International Cooperation Agency) and the University of Asuncion entitled: “*Estudio de tasa de contribución hídrica de las 4 subcuencas al Lago Ypacarai”* 4) tried to get a better view on the hydrologic regime of the 4 Investigación de las condiciones hidrológicas y geológicas de la cuenca del Lago Ypacarai, monitoreo de la cuenca del Arroyo Yuquyry. Informe final. Centro de Innovación Tecnológica. Facultad de Ingeniería UNA. Octubre 2005 33 Ypacarai hydrographic basin. Issues involved are the runoff discharge of the streams into the lake the discharge of the lake via the Salado river towards the Paraguay river and finally the so called Hydric Balance of the lake, both items are important when a biocatalyst might be applied.

The fieldwork has been carried out in the subbasin Yuquyry, as the Yuquyry stream is heavily polluted; the course of the stream follows through many built up areas before discharging into the Ypacarai lake. Rulers have been installed on selected locations along the Yuquyry stream. During many months the water levels and flow velocities have been recorded to determine the discharges in m3/sec.

Measurements have been carried out during more dry and wet periods. From this the Q/H relation (by regression analysis) is calculated. This study is important as from it the reaction on rainfall in the stream basin both in quantity and time (the hydrogram) can be defined.

The most important ruler/ flow station has been installed at the downstream end of the Yuquyry stream just before entering the wetlands. Wetlands possess a regulating action, both in biochemical sense (phyto-purification) and as buffer storage.
After a year of measuring (both during dry and wet months) sufficient pluviometric and hydrometric data have been recorded to define the so called Runoff Coefficients of the Yuquyry sub basin. On the basis of the Yuquyry sub basin Runoff Coefficients the same coefficients of the other sub basins have been determined to get the total Ypacarai Basin Runoff coefficients.

The latter is in the opinion of Wise Use rather tricky as this deduction has rather some uncertainties and required many assumptions, see the chapter “Conclusions”.
The calculation of the coefficients in the Yuquyry Subbasin defines, as stated, which part of all rainfall (stormwater) contributes to the wetlands and ultimately to the lake.
However the extra waste water, coming from the population and the industries, and discharged to the stream and its tributaries is not accounted for. There are estimates, but not accurate enough to be taken into account. The assumption is that the extra quantity (polluted water, wastewater) will be 10% of the total runoff at maximum. This means that the majority of the polluted wastewater in the wetter period will be rather diluted, but in the wet months will have a quite high degree of pollution.

It is estimated that the industry uses and discharges actually some 10 million m3/yr; a large part being untreated. The domestic wastewater is, in terms of quantity, the largest as it wi9ll be several millions of m3/yr.
The other sub basin (the Pirayu stream) flows through a significant less built up area, but carries more sediments, silt and clay (both in suspension and bottom load). The organic content of these sediments is not known, but as the Pirayu flows through a wetland before entering the lake the organic content will be rather big. This means that not all of the layer of organic (mostly anaerobic) sludge, sedimentated onto the lake bottom, originates from polluted wastewater.

The Runoff Coefficients
The pluviometric data are gathered from 3 gauge stations, one inside the Ypacarai hydrographic basin and two outside. In order to calculate the average rainfall in this basin the “Polygon Method” from Thiessen has been used. 34

Rainfall and runoff analysis during the more dry period
The total volume of the Yuquyry stream water that reaches the wetlands and finally discharges into the lake has been calculated by means of the recorded water heights, the discharges and the known rainfall data . This produces the following:
The total runoff during the more dry period (June through September) Vrdr is calculated at:
Vrdr= 646.324 m3 On the basis of pluviometric data (a theoretical approach) the rainfall for the entire Yuquyry basin can be calculated as: **Vndr= 6.833.787 m3**
The **Runoff Coefficient** during the drier **period** (June through September) is now:
Cdr = **Vrdr**
= 646.324 = **0,095** (June through September)
Vndr 6.833.787

Rainfall and runoff analysis during the more wet period
The total volume of the Yuquyry stream water that reaches the wetlands and finally discharges into the lake has been calculated by means of the recorded water heights, the discharges and the known rainfall data. This produces the following: The total runoff during the more wet period (October through May) Vrdr is calculated at:
Vrnat= 2.582.447 m3 On the basis of pluviometric data (a theoretical approach) the rainfall for the entire Yuquyry basin can be calculated as: **Vnnat= 16.643.108 m3**
De **Runoff Coefficient during the more wet period** (October through May) is calculated at:
Cnat = **Vrnat**
= **2.582.447**
= **0,155** (October through May).
Vnnat 16.643.108
Analysis of both scenarios:
1: A relatively dry year
Sub basin Aº Yuquyry

Table 4-1 Runoff coefficients dry year

Table 4-1 Runoff coefficients dry year

Period	months	rainfall (mm)	Runoff Coefficient	Area (km2) subbasin Yuquyry	Volume of water (m3)
dry	8	50	0,095	302	12.104.078
wet	4	150	0,155	302	29.049.788
				TOTAL	**41.153.866**

A yearly volume in a relatively dry year of **41.153.866 m3** for the Yuquyry stream means an average discharge of **1,32 m3 /s**. and: This would roughly indicate the following for the entire Ypacarai basin: A yearly volume of **113.844.960 m3** in the Ypacarai Hydrographic basin and thus an average yearly discharge of **3,61 m3 /s**.35
1: A relatively wet year Sub basin Aº Yuquyry

Table 4-2 Runoff coefficient wet year

Table 4-2 Runoff coefficient wet year

Period	months	rainfall (mm)	Runoff Coefficient	Area (km2) subbasin Yuquyry	Volume of water (m3)
dry	5	75	0,10	302	11.347.573
wet	7	225	0,16	302	76.255.693
				TOTAL	**87.603.266**

A yearly volume in a relatively wet year of **87.603.266 m3** for the Yuquyry stream means an average discharge of **2,81 m3 /s**. and:
This would roughly indicate the following for the entire Ypacarai basin: A yearly volume of **239.542.270 m3** in the Ypacarai Hydrographic basin and thus an average yearly discharge of **7,68 m3 /s**.

Remarks and conclusions by Wise Use on above stated study and results

Remarks:
Suppose the volume of the water body of the Ypacarai Lake (12 * 5 km and. 2 m deep!!) is estimated at 120.000.000 m3 . According to above stated rainfall data and runoff coefficient all the water of the Ypacarai Lake could theoretically be “renewed” in one (1) dry year.

In practice this will not happen due to the following factors:
- Not all the water that enters the wetlands discharges really into the lake.

- The infiltration and evaporation of the smaller (eastern and western) sub basins may reduce substantially the net discharge to the lake.

- The linear correlation between the Yuquyry sub basin and the other sub basins of the Ypacarai Basin has its limitations (will not be linear) The dendritic pattern of the Pirayu stream and tributaries is quite different than that of the Yuquyry stream; the hydric contribution to the lake will be less than theoretically adopted in the study.

- During periods of heavy rainfall the water levels in the wetlands may temporarily be high without a direct discharge to the lake.

- The evaporation downstream of the discharge measurement stations (in the wetlands before entering the lake) may be high during the year, up to more than 10% of the recorded flow volume.

- The geometry of the Ypacarai Basin may slow down the inflow of the rain water (the hydrograms). Especially during the drier period the (not saturated) subsoil is more suitable for infiltration.

- The calculation of the discharge of the Pirayu stream into the lake by correlating with calculated factors form the Yuquyry stream (thus without in situ measurements) may provide figures that are far too high. The differences in geomorphology between both subbasins are substantial (runoff patterns, slope and subsoil).

The Ypacarai Lake is situated in a geological depression, probably due to an existing fault. Of course this depression will be filled in by sediments (silt and clay) originating from the basin (hinterland). To maintain this lake as such some hydraulic control works are necessary. Beside the stated sediments a rather thick layer of organic sludge is present 36 on the lake bottom. This black sludge (sometimes very sticky) originates partially from former lake plants (when the lake was in a more healthy condition), partially from the wetlands and the industrial and domestic (organic) waste (suspended and then sedimentated).

The mostly contaminated anaerobic sludge could maybe be reactivated by the biocatalyst, after which parts will be emerged to the surface and will be ready for collection or dissolve in the lake water.

Conclusions:

1. The model presented shows that both in a dry and in a wet year the rainfall in the Ypacarai basin might be sufficient for a complete renewal of all the lake water body. As above stated this (theoretical) conclusion is incorrect due to the many factors that influence the yearly discharge volume.

2. Internal currents within the lake are not well known:
- the horizontal current (wind driven).
- The vertical or upwelling currents by for instance differences in temperature and concentration.

3. The present study (2005) from the UNA and JICA indicate that a cleanup of the complete Ypacarai Lake water body by natural renewal (auto regeneration by rainfall in the basin) plus the contaminated bottom sediments will take a long time. The auto regeneration might only then be successful if the polluted water inflow can be stopped.

4. The application of a biocatalyst (if successfully tested) for both lake water and lake sediments is necessary to generate the necessary biochemical boost for recuperation. However this only will be successful if small “treatment plants” or treatment stations are set up where the stream water receives it first boost (located at the downstream end of the stream, but before entering the wetlands). Even a normal aeration will help, but combined with a biocatalyst the first “drive” will be much more effective.

5. This first Yuquyry treatment station might be located at the crossing of the road (connecting the “compañías de Yuquyry (Luque)” and “Valle Pucu (Aregua)”) and the Yuquyry stream.

Whenever it is decided to divert (temporarily) the downstream end of the Yuquyry stream straight to the Rio Salado (and then to the Rio Paraguay) one has to bear in mind that this could mean a partial destruction of the wetlands (north of the Ypacarai lake). This idea should be carefully elaborated and analyzed and for sure is in an environmental sense not the favorite option.

As stated above: This first Yuquyry treatment station might be located at the crossing of the road (connecting the “compañías de Yuquyry (Luque)” and “Valle Pucu (Aregua)”) and the Yuquyry stream. Some historical data on discharges and velocities are given below. As one might notice, these values are somewhat higher than the recent calculated ones. The measurement apparatus and calculation method are probably somewhat different. 37

Table 4-3 Historic data discharges
More recent data by the mentioned report of 2005 (see footnote 1)

Table 4-3 Recent data discharges

Table 4-3 Recent data discharges

	INFORME UNA - JICA 2005				
	Date	Width M	Area Total m2	Total Discharge m3	Mean Vel. m/sec
	17-1-2005	6,60	4,13	2,22	0,54
	11-2-2005	6,00	2,85	1,11	0,39
	23-2-2005	6,70	2,84	1,08	0,38
	11-3-2005	6,50	3,01	1,02	0,34
	5-4-2005	7,60	4,48	2,37	0,53
	21-4-2005	7,60	8,33	2,86	0,34
Thunderstorm	17-5-2005	56,00	34,54	23,44	**0,68**
	10-6-2005	7,60	3,75	1,33	0,39
	13-7-2005	7,20	3,78	1,68	0,44
	4-10-2005	7,20	3,82	1,70	0,45

These data once again prove one of the hydraulic “laws” that the mean or average velocity in a non-confined flow depends largely on slope of the valley. The data shows that even with high discharge due to an intense thunderstorm the average velocity does increase, but reaches a critical velocity (Reynolds /Froud) after which it inundates the margins.

4.2.2 DESCRIPTION CONTROLLED TESTS WITH POCO

The recent 2007 report from Rediex has been used as data source for the determination of the analysis of the current pollution. pollution. The main objective is the gather special required information, translate it o the technical equipment, bio catalyst needed and finally the running cost for the completion of the project i.e. clean up of Ypacarai Lake.

4.2.2.1 ANALYSIS SEDIMENTS AND WATER QUALITY

Historical Discharge data Puente Yuquyry

Date	Width m	Area m2	Discharge m3/sec	Average. Velocity m/sec	Date	Width m	Area m2	Discharge m3/sec	Average. Velocity m/sec
11-Dic-98	8,0	7,11	7,39	1,04	21-Jul-00	6,6	0 ,58	2,67	0,75
12-Mar-99	10,0	8,59	10,15	1,18	17-Ago-00	6,4	0,70	2,63	0,71
13-May-99	2,0	0,30	0,16	0,53	25-Ago-00	6,3	3,36	2,16	0,64
04-Jun-99	5,3	4,23	4,59	1,09	14-Sep-00	6,4	3,81	2,91	0,76
02-Sep-99	6,0	4,47	3,69	0,83	01-Dic-00	7,0	4,50	4,16	0,92
12-Oct-99	5,0	3,41	2,85	0,84	19-Ene-01	5,8	3,00	1,76	0,59
21-Oct-99	5,0	3,68	2,56	0,70	26-Ene-01	6,0	2,82	1,75	0,62
28-Abr-00	6,4	3,49	2,49	0,71	16-Mar-01	5,0	3,26	2,05	0,63
08-May-00	7,4	5,51	4,66	0,85	20-Jul-01	8,5	4,26	2,09	0,49
23-May-00	6,3	4,06	2,84	0,70	07-Sep-01	7,7	4,01	2,17	0,54
20-Jun-00	7,7	5,85	5,91	1,01	04-Oct-01	6,3	3,37	2,55	0,76
30-Jun-00	7,0	5,00	4,75	0,95					

Some existing data on sanitation within the Ypacarai Basin.

Table 4-4 Sanitation data

Community	Population within the Ypacarai hydrographic basin		Connected to Running water	Connected to sewage system	Septic tank / or sewage pit
San Lorenzo	184,867	Yuquyry Subbasin	26.81%	8.10%	59.80%
Luque	136,505		25.60%	11.60%	53.30%
Capiata	154,520		14.32%	0.44%	53.10%
Itagua	61,670		30.53%	0.00%	54.80%
Aregua	44,480		20.26%	0.00%	57.20%
J.A.Salvador	37,480		34.48%	0.00%	50.70%
Fndo.Lamora	19,367		87.27%	0.00%	80.00%
Guarambare	1,172		60.22%	0.00%	47.60%
Ita	3,674		17.29%	0.00%	37.20%
Ypane	645		25.22%	0.00%	50.70%
	644,380				
Ypacarai	18,780	Pirayu subbasin	40.03%	0.00%	56.20%
Yaguaron	3,426		21.50%	0.00%	46.00%
Paraguari	6,346		29.70%	0.00%	55.20%

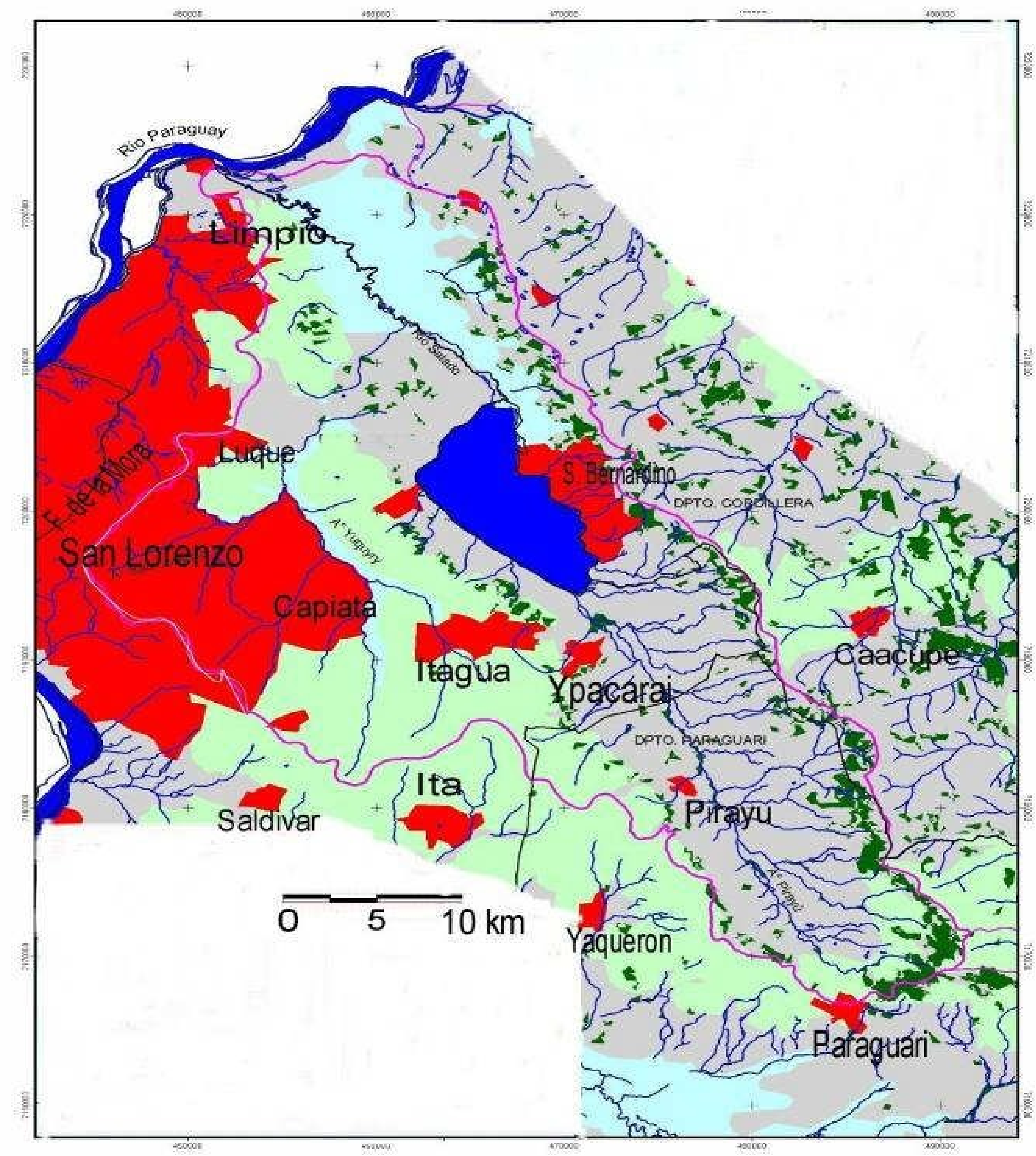


Figure 4-1 Ypacarai Basin

Striking is that around 25% of all people within the Ypacarai Basin are connected to running (tap) water. However only the bigger villages (those near the capital Asuncion) are connected to a sewage system. Given the above stated figures, this means that for domestic wastewater around 50% of the people have a septic tanks or sewage pit. In normal conditions this prevents the contamination of the streams like the Yuquyry. The only thing is: “what happens if the pit is full”; will it be emptied and centrally treated or just dumped ??

Available data from other sources:
JICA (Japan International Cooperation Agency) did in 2004 and 2006 some chemical analysis on samples from the Yuquyry Stream. Fig.4.2.2 shows how the Yuquyry stream and tributaries are flowing through built up areas, of which San Lorenzo and Luque and Capiata relatively quite populated, see table below.

Table 4-5 Population data

Table 4-5 Population data

Community	Population total	Population within the Ypacarai hydrograph basin	Population within the Yquyry Subbasin
Aregua	44,480	44,480	
Capiata	154,520	154,520	154,520
Fndo.Lamora	113,990	19,367	19,367
Itagua	61,670	61,670	61,670
J.A.Saldivar	37,480	37,480	37,480
Limpio	72,510	29,548	
Luque	185,670	136,505	136,505
Pirayu	14,860	14,117	
San Bernard.	9,550	9,550	
San Lorenzo	203,150	184,867	184,867
Ypacarai	18,780	18,780	
Total	**1,011,340**	**710,884**	**594,409**

This means that actually some 710.000 people live in the Ypacarai Basin. The industries involved are mainly slaughterhouses (cows and pigs) and the leather tanning industry. Table 4.2.2.3 shows an example of the volumes industrial wastewater (treated/untreated) that is discharged into the wetlands and finally into the lake (on a daily basis).

Table 4-6 Waste water discharges industry

Table 4-6 Waste water discharges industry

INDUSTRIES			Treated wastewater			Untreated wastewater
	With tr. plant	heads	m3/day	heads	Without tr. plant	m3/day
slaughterhouses	5	340	272			
slaughterhouses				678	15	860
Refrigerating industr.		800			1	1200
		skins				
Tanning	4	460	160			
Total:			**432**			**2060**

Apart from the industrial wastewater is the domestic wastewater. The table below shows an indication of the values concerned with domestic wastewater loads.

Table 4-7 Loads domestic discharges

Table 4-7 Loads domestic discharges

	DOMESTIC	loads (kg / dia)				Colif .fecal
Population in the Yuquyry Subbasin	**Population in the Ypacarai basin**	**BOD**	**Nitrogen**	**Phosfor**	**Solids**	**(X x 10^n)**
	44,480	1,179	266.9	33.6	2,669	4.5E+13
Capiata	154,520	6,181	927.1	123.6	9,271	1.5E+14
Fndo.Lamora	19,367	775	116.1	15.5	1,155	1.9E+13
Itagua	61,670	2,467	369.8	49.3	3,679	6.2E+13
J.A.Saldivar	37,480	1,499	224.8	30.0	2,236	3.7E+13
	29,548	1,182	177.2	23.6	1,763	3.0E+13
Luque	136,505	5,460	818.6	109.1	8,143	1.4E+14
	14,117	565	84.7	11.3	842	1.4E+13
	9,550	382	57.3	7.6	567	9.6E+12
San Lorenzo	184,867	7,395	1,109.0	147.8	11,027	1.8E+14
	18,780	751	112.6	15.0	1,120	1.9E+12
594,409	**710,884**	**27,836**	**4,264**	**566**	**42,472**	**7.E+14**

Figures (in yellow)discharge into the Yuquyry stream

Analysis of the water quality, both in the Yuquyry stream and the lake surface water have been carried out by JICA, and provide the following data (excerpted and assembled from non-published information)

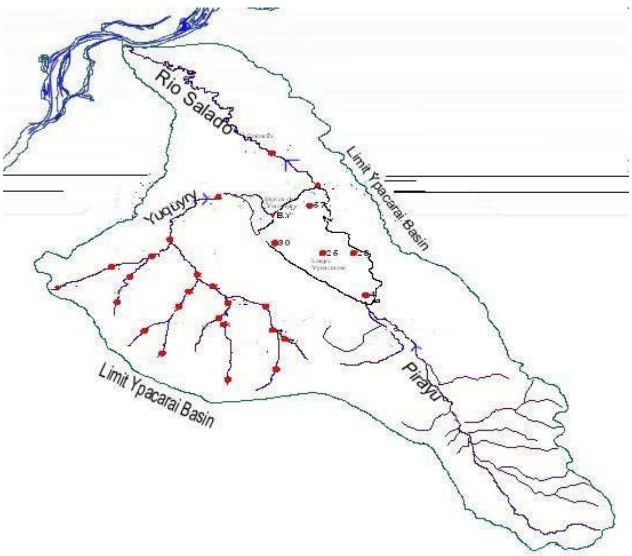


Figure 4-2 Sample locations Ypacarai basin

Parameter	Oxig. mg /L	BOD mg /L	COD mg /L	Phosph. mg /L	Nitrog. mg /L	Coli Fecal	
from 1995 - 2000		water quality of the principal streams.					
A.Yuquyry	5,2	5,5	45,5	0,57	3,71	6930	
A.Pirayu	8,0	1,2	41,1	0,09	1,31	9421	
Rio Salado	8,1	1,5	64,6	0,26	2,56	384	
Yuquyry stream July 2005 till February 2006							Solid.totales
Range of variation	2,6 - 6,1	4,2 - 7,8	-	0,31- 1,1	2,49- 7,28	400-8700	298- 392
Reference value	>5,0	<5,0	-	<0,025	< 0,3	<1000	

Table 4-8 Biochemical data water quality (I)

Measurements in the Ypacarai Lake					
Parameters / Points	4	25	28	30	57
P total mg/l	0,20	0,22	0,21	0,23	0,23
PO4 mg/l	0,12	0,13	0,18	0,13	0,12
Clorofila-a ug/l	21,0	25,0	25,8	31,8	30,8
Coliforms NMP	272	113	91	191	120

Table 4-9 Biochemical data water quality (II)

state	Tot. Ph mean concentration.	Chlorophyll-a	Secchi disk
	mg/l	(ug / l)	(m)
		Media máx.	médio max.
Hipertrophe	> 0,10	> 75	< 0,7
Ypacarai Lake	0,22	4 - 85	0,2 – 0,5
The Lake is in an Eutrophic to Hipertrophic state			

4.2.3 ANALYSIS OF HYDROLOGIC ASPECTS YPACARAI LAKE

There are two major inlets for the lake; one is situated in the north-western part of the lake (the Yuquyry Stream). This stream enters the lake via a wetland in front of the lake.

The Yuquyry entrance to the lake

The other inlet is situated in the southern part (Pirayu Stream), and enters also via a series of wetlands. The Salado River (at the north-eastern side) is the outlet of the lake and discharges into the Paraguay River. About 80 % of the total pollution is due to Yuquyry stream, carrying domestic sewer products as well as industrial waste water with derivatives from slaughterhouses, leather-, oil- and food processing industries.
The other 20 % of the pollution is due to the influx of the Pirayu stream, which is mainly responsible for the influx of sediments. Some parts of the lake are covered with weeds such as *Eichhornia crassipes* (Water Hyacinth); *Pisita stratiotes* (Water Lettuce) etc. These weeds reproduce through vegetative propagation and growth is greatly enhanced by high nitrogen, phosphorus and potassium levels.

This creates adverse effects on water quality and reduction in biodiversity which will result in depletion of dissolved oxygen. Reduction of dissolved oxygen creates direct impact on 42 the microbial activity of the micro-organisms resulting in incomplete degradation of the organic load & increase in the contamination levels. The top layer of the lake (about 0.5 meters to 1 meter) is probably aerobic and the bottom (biofilm) will be rather anaerobic. The natural contamination load in the lake water is low, but the Industries and domestice wastewater increase the pollution considerably (contamination load), in addition the lake depth has considerately been reduced over the many decades, mostly by sediment influx.

4.2.4 PRE-PESP STAGE: ANALYSIS OF SEDIMENTS AND WATER QUALITY

4.2.4.1 THE FIRST TESTS IN 2007 (PRE-PESP STAGE)

The sampling locations for the laboratory tests were carried out in February 2007 by Wise Use and CEMIT and selected around the Yuquyry stream i.e. from the highly polluted area and from those parts where the sticky black bottom sediments were present (at the . Considering the aim of the project i.e. pilot studies the samples were collected form the organically and inorganically polluted area, one point above and one below the point of discharge. The sampling tanks were rinsed thoroughly with the polluted water and the physiographic conditions were maintained to reflect lake conditions as far as possible.

Recollection and preparing of samples.

The composite samples (500 ltrs x 3 tanks) were pumped from the bottom and the middle part of the lake. These samples were carried to Multidisciplinary Centre for Technological Research at Asuncion National University Campus to design the experiments and to carry out the necessary analytical work.

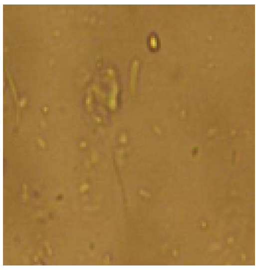
Immediately after the sample collection the first analysis were carried out. The results are depicted in Table 1 (0 hours).
The pilot experiment (500 ltrs x 3 tanks) was designed using batch mode process and the aim is to confirm the efficacy of the biological stimulator i.e. PoCo (Pollution Control) in both aerobic and anaerobic conditions. Since the dissolved oxygen content in the samples was very low at start, i.e. almost less than 0.2 mg/L, it was necessary to obtain and maintain the dissolved oxygen content at an optimum level in tank I and II (aerobic environment); to increase the amount of micro-organisms which will help to achieve faster degradation of complex-organic molecules (lodo). The dissolved oxygen content in the tank I and Tank II was maintained at an acceptable level with the aid of air-compressor. In tank III (anaerobic condition) manual agitation was necessary to avail the nutrients present in the organic sludge (lodo) and activate the growth of micro-organisms.
The details for the pilot experiments are as follows:

Pilot Experiment I (3 tanks x 500 ltrs)
Tank I - 500 ltrs sample + Aeration
Tank II - 500 ltrs sample + Aeration + **PoCo** @ 10 mg/lit
Tank III - 500 ltrs sample + No Aeration (i.e. maintaining a more Anaerobic state) + **PoCo** 10 mg/lit + Manual Agitation for 5 min @ interval of 2 hrs

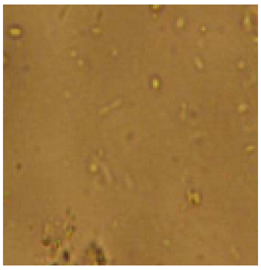
Note :
Tank II - **PoCo** was added @ ‘0’ hrs
Tank III - **PoCo** was added after ‘36’ hrs in anaerobic condition
The above set of experiment was continued for 5 days followed by continuous aeration for tank I & tank II, while tank III was followed by manual agitation only. The samples were analyzed for ‘0’ hrs, ’48’ hrs and ‘120’ hrs, the results for the same are as follows:

Table 4-10 Pilot Test series I (3 tanks x 500 ltrs) :

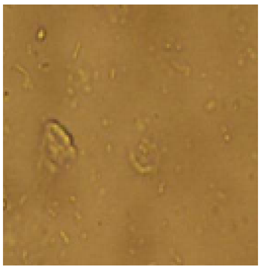
Sr. No.	Parameters	Unit	Tank I			Tank II			Tank III		
			'0' hrs	'48' hrs	'120' hrs	'0' hrs	'48' hrs	'120' hrs	'0' hrs	'48' hrs	'120' hrs
1	pH	---	6.27	7.23	7.07	6.42	7.13	7.06	6.18	7.10	7.06
2	Color Real	Hazen	1500	1500	1000	1800	1000	1500	1500	1500	1500
3	Odour	---	Bad	Mild	No	Bad	Mild	No	Bad	Bad	Bad
4	Turbidity	NTU	1160	115	630	440	180	660	420	860	630
5	Conductivity	µS/cm	188	177	173	192	183	181	177	248	269
6	Temperature	°C	27.9	23.2	22.6	26.4	23.9	24.3	26.2	24.5	25.1
7	Dissolved Oxygen	Mg/L	0.14	6.20	8.05	0.13	7.66	6.52	0.16	0.08	0.06
8	BOD @ 5 days	Mg/L	101	Not Done	Not Done	100	Not Done	Not Done	117	Not Done	Not Done
9	COD	mg/L	6335	544	4050	5585	831	3090	5041	5017	3521
10	Nitrates	mg/L	0.127	0.802	0.461	0.330	0.359	0.685	0.265	0.144	0.332
11	Phosphates	mg/L	12.4	0.107	5.57	7.65	0.158	5.76	9.55	5.09	5.63
12	Sedimentable Solids	ml/L	66.0	9.0	57	58.0	10	50	81.0	53	50
13	Suspended Solids	mg/L	23830	1080	9300	13638	1760	9560	12530	12060	9460
14	Dissolved Solids	mg/L	2718	400	225	2703	450	1000	2943	750	1100
15	Total Coliforms	Cfu/100 ml	1300	1000	200	900	100	1400	200	1200	3200
16	Fecal Coliforms	Cfu/100 ml	0	0	0	100	0	0	0	0	0



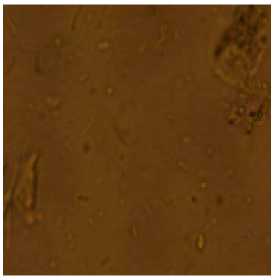
Tank I - Foam



Tank I - Foam



Tank I - Water

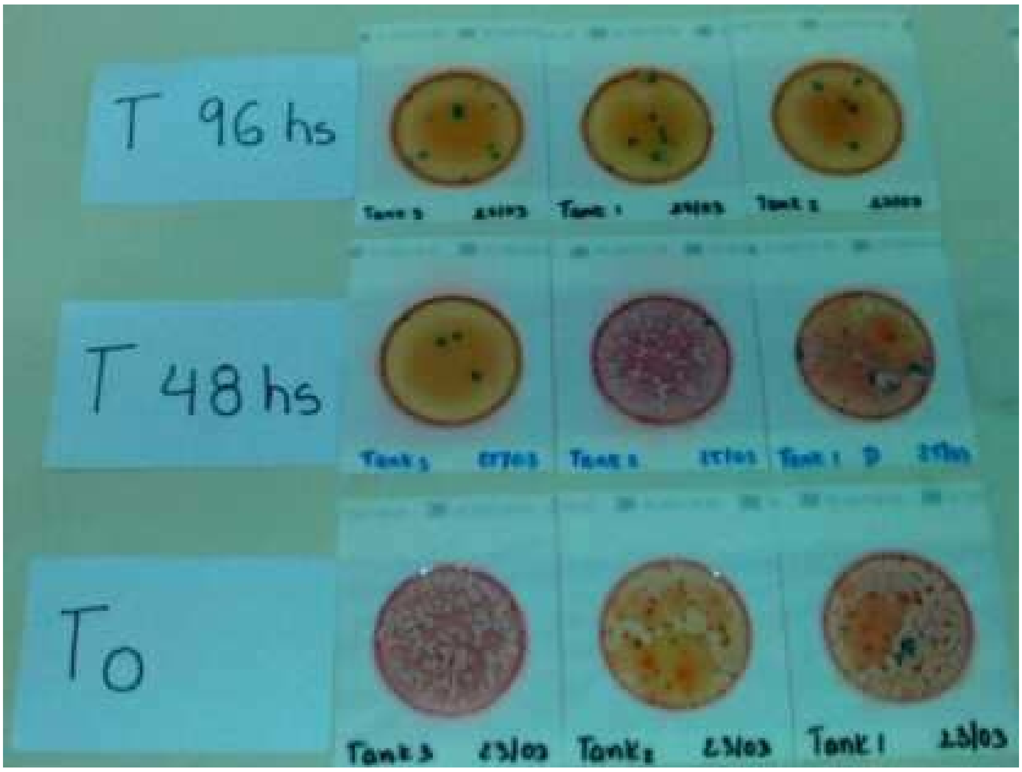


Tank I - Water

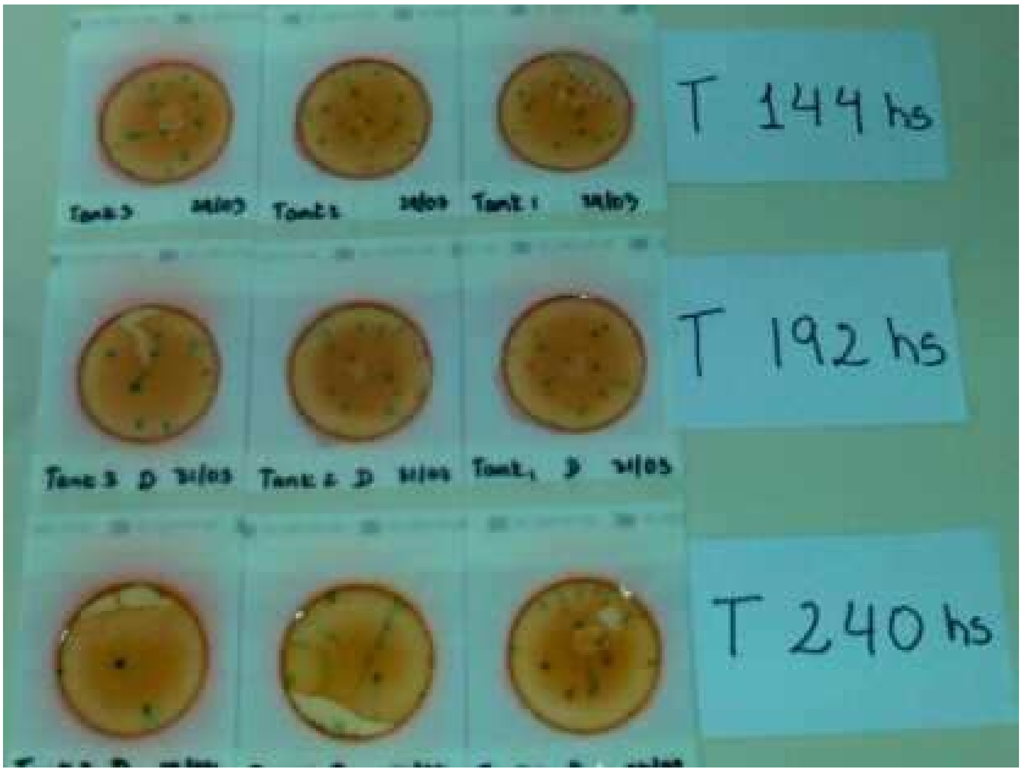
Microscopic Examination of Foam & Treated Water for Tank – I was done in order to check out the contamination level and the bacterial count. All the samples showed various shapes of bacteria viz; Bacillus, Coccus, Spiral & Cocci in Chain.

The bacterial cell count was 1 x 108 cells/ml and above, while the Vitality (live cell percentage) was more than 85 % when checked with Haemocytometer. The microscopic examination proved that the no. of micro-organisms and their activity is quiet enough in the process to degrade the organic matter though the DO levels are low.

Microscopy results for Total Coliforms are as below **:**

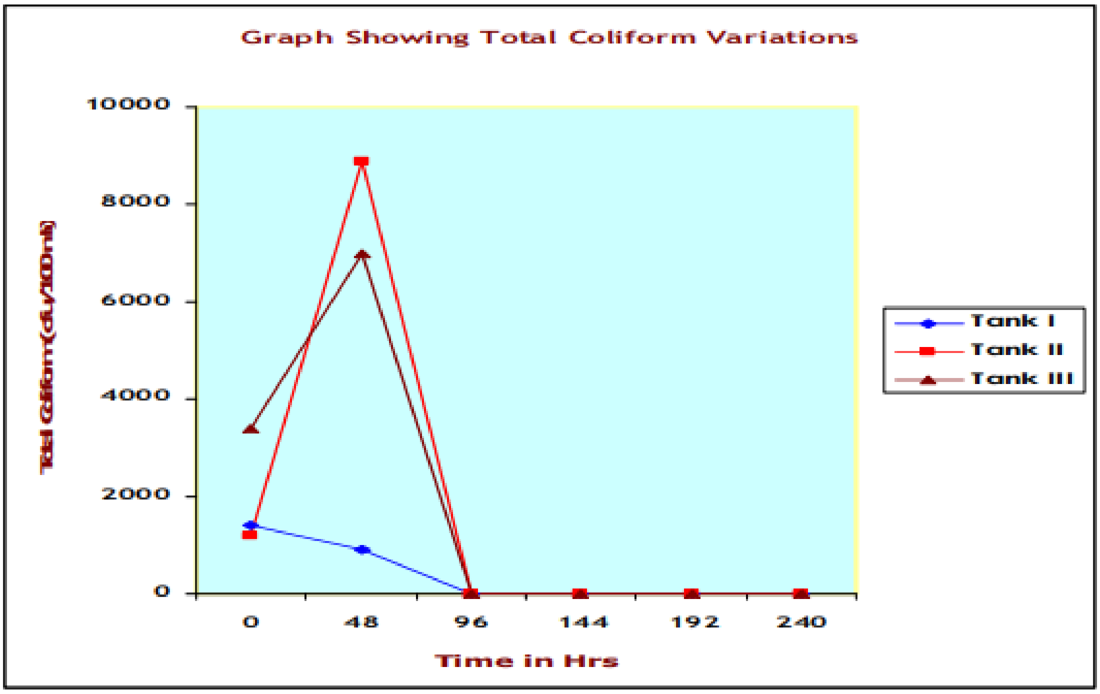


Time – '0', '48' & '96' hrs



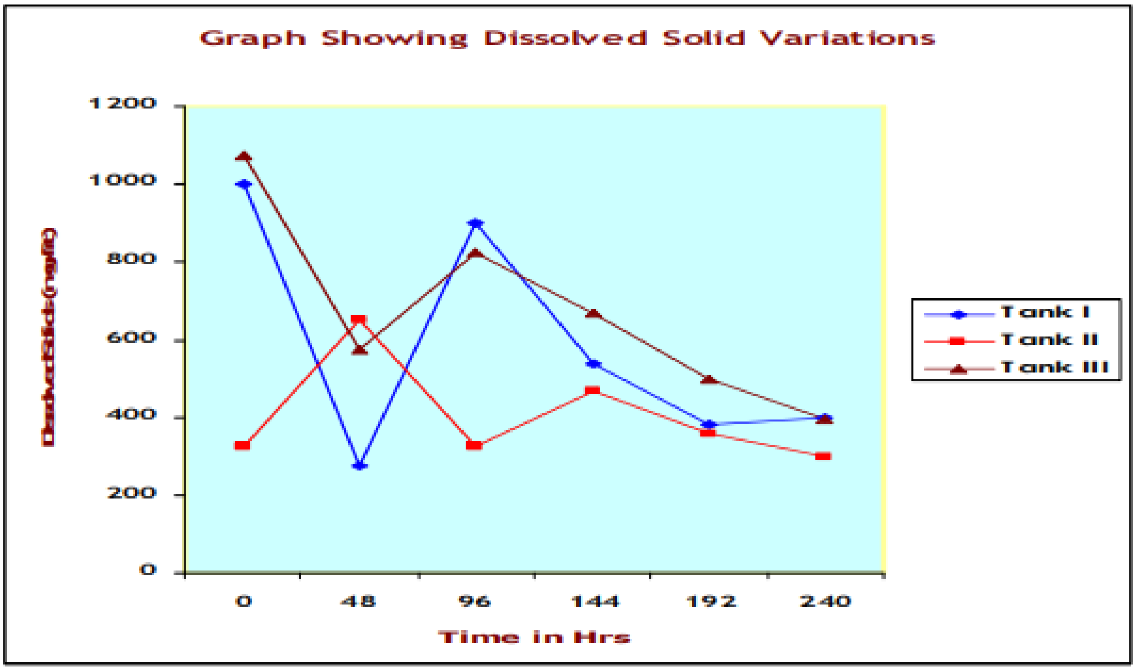
Time – '144', '192' & '240' hrs

Figure 4-3 Total coliform variations



The results for Total Coliforms & Fecal Coliforms after '96' hrs are fine & very good i.e the concentration is almost nil. Generally Total Coliforms & Fecal Coliforms results are observed after '24' hrs or '48' hrs incubation & if the same results are observed after '72' hrs incubation or more, this may give false results due to contamination or over incubation. So it's very necessary and important to monitor the results at the specific time.

Figure 4-4 Dissolved solid variations



Dissolved Solid concentration is also lower in anaerobic one when compared with other two.

Conclusion of Pilot Test Series 1:
1: Key factor for life of micro-organisms is 'oxygen'. Both in tank I and tank II oxygen is added (through passage of air). After 48 hours the levels of COD, Coliforms and Dissolved Solids decrease considerably. This proves that the activity of the micro organisms was good which consumed a lot of organic stuff/matter from the wastewater. Odour reduction was also observed along with reduction in Colour i.e. the water was looking more transparent as the time passes.
PoCo helped micro-organisms to maintain lower COD levels in tank II than in tank I. The dissolved oxygen level after 48 hours observed higher along with faster smell reduction and the observations were constant for longer time than the tank without PoCo.

2. Normally the maximum activity of micro-organisms is observed when the dissolved oxygen is maintained between 2 and 4 Mg/L. In this first test the dissolved oxygen was much higher than the optimum one so the activity of micro-organisms was increased much faster and consumed all the possible food/nutrients from the specific amount of water quantity, this resulted in decrease of micro-organism population in tank I and tank II almost over after 120 hours. The micro-organisms population was higher in tank II as compared to tank I thus can be concluded from the analysis report. The micro-organisms have 'consumed' all the food and later due to starvation they were not able to survive anymore, this resulted in raise of the solids in the tanks.

In tank III (anaerobic condition) we can observe that the micro-organisms activity is improved due to PoCo which resulted in reduction of COD levels from 0 until 120 hours and simultaneously production of methane and nitrogen.
Remark: Scientifically the conclusion for the First Laboratory Set is almost nil due to the misbalanced of nutrients/food. It is difficult to confirm the efficacy of PoCo based on these results so we need repeat the test in situ in order to obtain reliable data. However, it has been concluded that 'something' happened after addition of PoCo to the tanks. Based on this the idea of upscale test in the swimming pool of the Nautic Club originated and was carried out by Specialized University Department CEMIT (Paraguay) with collaboration of WUR (Netherlands).

4.2.5 PREPARATION SECOND TEST UNIVERSIDAD NACIONAL (CEMIT)
CEMIT does not sufficient equipments for sampling, instruments for analysis, man power etc so Wise Use managed a team of 3 persons to carry out the necessary work and was informed about the PoCo application and the parameters to be measured. This work was carried out under supervision of the Head of the Biochemical Department of CEMIT, Dr. Inocencia Peralta.

4.2.6 IMPORTING CONCENTRATES & ADDITIVES IN ORDER TO PRODUCE POCO
To overcome the problems such as delays in International Transport, Custom Clearance etc the PoCo Samples were sent by Courier Service saving valuable time.

4.2.7 MANUFACTURING F10 DOSIFICATION

In the CEMIT laboratory, F1 concentrate was added to heated and cooled purified water and after adding additives/preservatives the ready to use product was stored in jerry cans in a refrigerated room. This product was used for the second test to be conducted in the swimming pool and the dosage of the product was as 1 liter of concentrated PoCo for 1,000 m3 lake water.

4.2.8 PREPARATION OF ARTIFICAL POOL

Club Nautica has 2 swimming pools. One has an open connection with the lake and the other pool is closed but has a pumping station and sand filter that renews the water at given intervals.

Pool 1 **Pool 2**

The first test was executed in Pool 1 where the conditions (water and water bottom, sludge) are similar to the adjacent Ypacarai Lake. The pool was closed by building metal dam board around the pool. Then pumps were installed and all connections for pipes, electric cables and breakers were made.

4.2.9 CONDITIONING WATER ENTRANCE AND OUTLETS WITH PROFILED METAL DAM BOARDS

As can be seen on the photos of pool 1, a walking bridge forms the boundary of the pool with the lake. The water can freely enter and exit the pool. This was closed before any testing could start. As stated above, a (metal) sheet wall was attached tot he walking bridge and hammered deep enough into the pool bottom sediments.

Open connection lake and pool **Installing dam boards**

Connection with the lake was closed

4.2.10 INSTALLATION OF THE PUMPS

Pumps were installed on two different locations creating some current in pool 1. This ensured that Poco was uniformly dispersed.

Preparing PoCo Pool 2: **addditional pumps and a sprinkler**

installation

4.2.11 WATER ANALYSES FROM MARCH 11 TILL MAY 16 2008

The test was performed in a closed pool located at the *Club Náutico San Bernardino*, of approximately 40 x 40 meters and 2 meters of depth, with a volume of nearly 3000 m3 . The thickness of the biofilm at the bottom (sludge) was measured at some 40 cm and resembled that of the lake. After introducing PoCo, water samples were taken on a daily basis from March 12 to 23 and then weekly from March 25 until May 7. Some 100 grams 50 of the bio-catalyst were added daily from March 12 to 18, then 200 grams from March 19 to 25, and again 100 grams from April 2 to 15, and lastly 200 grams from April 16 to 20 and from April 21 to May 7.

The analysis were performed based on Standard Methods for the analysis of drinking- and waste water (Standard Methods, 20 Ed), for physical, chemical, and bacteriological parameters. The complete testing results can be found in the annexes.

4.2.12
CONTINUED SAMPLING AND CONTROL FOR 60 DAYS, WEEKLY INTERVAL

Addition of PoCo and Sampling was done by the CEMIT team, pictures below shows the sampling techniques and on site measurements carried out by CEMIT.

PH measurement

sediment for lab analysis

Oxygen measurement

testkit

4.2.13 OBSERVATION FLORA & FAUNA,

Test length and duration was not sufficient to study flora and fauna or to undertake special observations. Prior to starting the test in pool 1, both CEMIT and SEAM (Ministry of the Environment) expressed their concerns on the possibility of PoCo leakages to the lake which might affect its natural environment..

SEAM sent an inspector to Club Nautica to verify that the pool connection to the lake was closed and gave the green light to commence the test series. At the start of the test, the pool water was green of algae and living fish were not present. It was decided to put new fish in the pool, i.e. the species *tilapia*. During the test period no dead fishes were found in the pool. In contrast, many fish offspring could be seen in the water in addition to a lively activity of the fishes in the water.

Example of fish offspring

Conclusion after finishing the test (16th of may 2008):

The complete results of the test and analyses made by CEMIT are included in the annexes. The quite opaque water changed to be a little more transparent (secci disk) with the start of adding PoCo (degradation / flocculation and sedimentation of the suspended load, followed by an increase due to the microbial activity in sediments, resulting in re suspension. The COD did not show significant variations throughout all stages of the test. The increase in total phosphor during the second stage might have been due to the increase in degrading micro-organisms of organic matter, thus releasing phosphor as organic form, and also due to the change from acid pH to alkaline.

- With regards to total colyforms, an important decrease has been observed after adding the product.

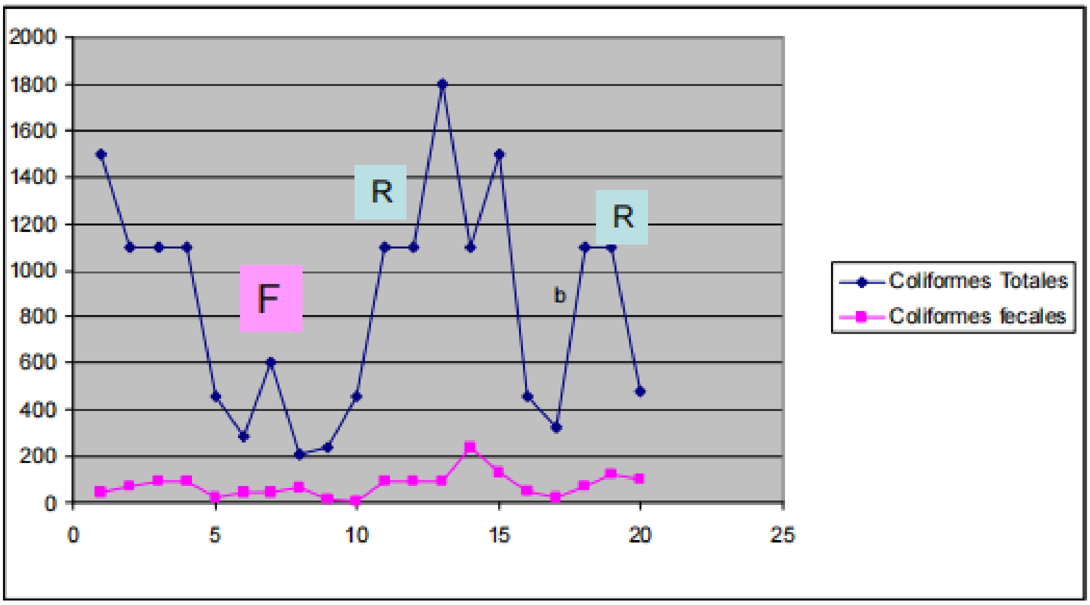
- It is worth to point out that in all stages of the treatment, there was an important decrease over the cyanobacteria population by adding the PoCo, which is of great importance, for one of the most serious problems of the lake is the degree of eutrophization.

The zooplankton population, specifically the copepods one, increased due the higher biodiversity of nutrients caused by the decrease of cyanobacteria and the increase of the other divisions.

Remark:

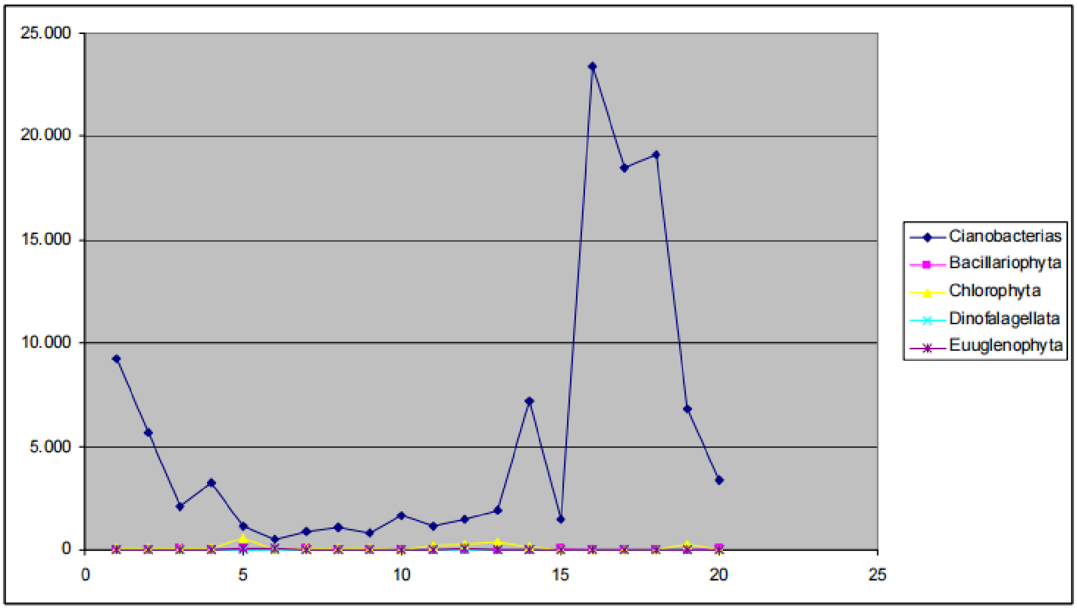
It is important to highlight that the most important parameters for MOPC are the decrease of cyanobacteria and coliforms. The first aim of cleaning up the Ypacarai lake is to get it **SAVE for recreation**. According the results of the second test, there can be observed that water quality has improved considerably from category 5 (recreation not permitted) into 2 (save for recreation)

Figure 4-5 Coliformes period



Coliformes period: 11/03/08 – 30/04/08

The above graph shows the decline of Coliforms from 1500 to 200. The metal sheet dam board ruptured (marked with R) because of natural disaster i.e. stormy weather and wave action on the lake. Due to the rupture of metal sheet dam the untreated water outside the pool mixed with the treated water in the pool this resulted in increase of Coliforms which reached upto 1800. Further the metal sheet dam was reconstructed and the water was again treated with PoCo which drastically resulted in decease of Coliforms to 300 in a short specific time. The same thing happened second time i.e. rupture (also marked with R) and reconstruction of metal sheet dam which resulted the ups and downs in the Coliforms count but finally we achieved low count after addition of PoCo. The behaviour of Coliforms proved that PoCo effectively helps to brings down the Coliform population in short specific time.



The same case is observed with the blue algae and cyanobacteria too. From day 1 after addition of PoCo reduction of the cyanobacteria is observed to acceptable levels. After the rupture of dam (day 15) and due to the mixing of untreated water to treated water again the cyanobacterial population increased but after reconstruction of dam and addition of PoCo again the cyanobacterial population was down to the acceptable level. This proved that PoCo helped a lot to bring down the cyanobacterial population same like coliforms provided that the untreated water is not mixed with treated water.

5 MARKETING OBJECTIVES

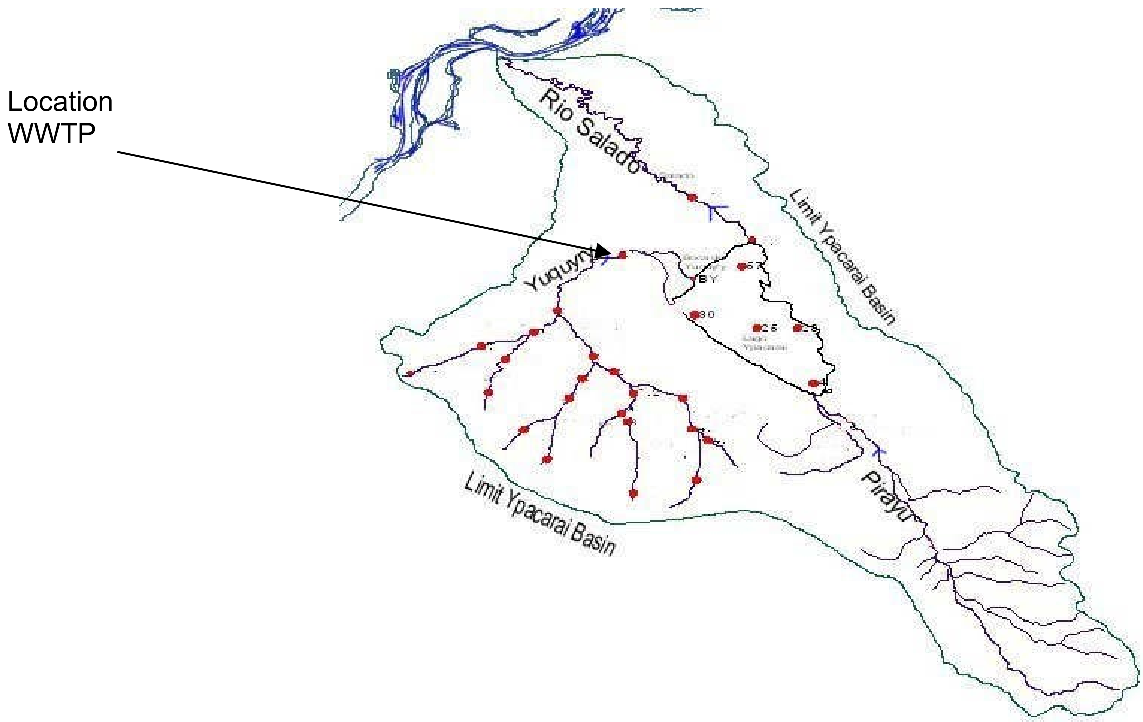
5.1 PREPARATION OF COMMERCIAL PROPOSALS

Various companies were selected on the basis of experience amd knowledge of the required materials and supply the equipment for the lake's problems. Handels buro Visser coordinates all proposal applications from various Dutch suppliers and consolidates them in one package. The proposal includes the engineering, supply and commissioning of all hard ware and is included in the annexes. The proposals contain the following key components.

1 Small Waste Water Treatment Plants (WWTP)

The first proposal entails the installation of a specially designed Waste Water Treatment Plants (WWTP) in the Yucuyry stream before entering the wetlands. The installation will be designed according to the pollution degree and the river current or discharge (see chapter 4.2.2.). The principle is based on biological phyto treatment. At present the actual treatment is done by the wetlands themselves, but their buffer function is limited after decades of pollution and sedimentation of floculated particles.

Figure 5-1 Location WWTP



Measurements suggest its counter-effect is negligible. Both input and output needs to be controlled enabling the treatment of the water in the biological treatment installation. Its effect is based on patented aerated installations and the addition of PoCo as a bio catalyst. Envisaged key supplier is the Dutch company Bosman Water management which also uses additional suppliers for mixing and dosing of PoCo. The price of the biological WWTP is substantially lower as compared to a conventional WWTP. Capacity is limited but given the low water discharging in the stream an investment in a large and expensive WWTP is not justified. The commercial offers from Bosman and Frits Visser Handels & Adviesbureau are attached herewith for study. The dimension of the WWTP shall be calculated by Paraguayan engineering companies. The exact dimension depends on the average discharge of the Yuquyry stream. Based on information obtained of the UNA and 54 JICA reports, the best place to build the WWTP is the location as depicted in the above figure. This is just before entering in the wetlands where the stream is split into several small streams. Exact location depends also on the availability of existing roads, electricity etc. The data used for the cost indication is based on the JICA report and REDIEX information.

Basic calculation:
Separate Module of Bosman WWTP exists of 100 aeration plates. These plates have a capacity of 80m3/hour. The dimensions of the module are 2x1m.
The average current of the Yucuruy river is:
1,32m3/sec in the dry season
3,61m3/sec in the wet season
Average for calculation: 2,47m3/sec (8874m3/hour)
Quantity of Bosman modules of 80m3/hour to be implemented: 110
Price of each module: € 195.000
Total price indication: € 21.450.000

Bio lava filters

35 bio lava filters will be installed in industrial wastewater loads and various small currents discharging into the lake. Lava is a porous material with a high internal surface. Pollution is retained in the lava filters through aeration, activation and acceleration of micro-organisms with the addition of PoCo and finally the water is treated. The treatment capacity of these filters is substantial and the solution seems cheaper than pumping the water to the WWTP.

Small currents normally are not polluted. Industrial loads have higher priority for implementation of a BLF (Bio Lava Filter). The JICA report shows a list of Industrial loads.

Table 5-1 Industrial loads

INDUSTRIES			Treated wastewater			Untreated wastewater
	With tr. plant	heads	m3/day	heads	Without tr. plant	m3/day
slaughterhouses	5	340	272			
slaughterhouses				678	15	860
Refrigerating industr.		800			1	1200
		skins				
Tanning	4	460	160			
Total:			**432**			**2060**

For more details, check the attached offer.

Poco Supply Units (PSU)

These installations will be installed at 5 days distance from the discharge into the lake. It contains the dosing pump that injects PoCo in determined quantities into the lake. The current ensures that sufficient oxygen is added to the process, COD5 (chemical oxygen demand) could be, and BOD5 (biological oxygen demand) is, influenced positively by PoCo. The objective of these PSU's is to boost the biochemical capacity of the WWTP's.
In chapter 4.1.2. a flow diagram is shown. The PSU basically can be implemented in small currents to pre-activate the water with PoCo. The PSU is far cheaper than the Bio Lava Filter, but the efficiency will determine whether the implementation of a PSU or BLF will be chosen. The indicative price setting for a PSU is € 30.000. The maximum capacity is 30m3/hr.

Cleaning the lake water body

Only after the daily discharge of polluted water into the lake has been addressed and contained, the actual cleaning of the lake can commence.
The cleaning includes the water and sedimentation. Previous proposals to dredge the sedimentation from the lake bottom were too expensive. However, this option is valid for some of the beaches. By removing the sedimentation and by nourishing new sand on the beaches, attractive beaches can be created. MOPC has a small dredging installation that can be used for this.
The Poco alternative was received positively as it cleans both the water and the sedimentation. The question arose how Poco could be distributed in an cost effective way. Natural currents are an options but it would take about a year to get to 30 km distance. This may be enhanced by a flat bottom that can be revamped to distribute Poco. Considering the tourism attractiveness the use of tourist flat bottoms actually used in Amsterdam was discussed. De Kooi in Amsterdam has relatively cheap second hand flat bottoms on offer.

The main pollution concentration (polluted lake bottom sediments) is located in the middle of the lake due to the internal currents and the geometry of the lake bottom. On these locations (middle of the lake) a floating structure will be placed with a generator, fuel tank and compressor. The aim is to enhance the natural cleaning capability by addition both PoCo and oxygen. Handels and Advies bureau Visser will supply all equipment while MOPC will supply the floating structure. The offer of the boat is attached.

Calculation quantity of PoCo needed:
At first a separation has to be made in A): cleaning the lake and B): maintaining it clean.
A: Initial investment
The volume of the water body of the Ypacarai Lake (12 * 5 km and. 2 m deep!!) is estimated at 120.000.000 m3 .
According Wise Use, 1 liter PoCo is needed for 1.000m3 of water. Theoretically we need 120.000 liter of PoCo to clean the existing situation at €70 /liter. The total amount of PoCo will then be approximately € 8.400.000
B: Running Costs
According to stated rainfall data and runoff coefficient (JICA 2004 report) all the water of the Ypacarai Lake could theoretically be “renewed” in one (1) dry year.

1: dry year

Table 5-2 Runoff coefficients dry year

Period	months	rainfall (mm)	Runoff Coefficient	Area (km2) subbasin Yuquyry	Volume of water (m3)
dry	8	50	0,095	302	12.104.078
wet	4	150	0,155	302	29.049.788
				TOTAL	**41.153.866**

A yearly volume in a relatively dry year of **41.153.866 m3** for the Yuquyry stream means an average discharge of **1,32 m3 /s**. and:
This would roughly indicate the following for the entire Ypacarai basin: A yearly volume of **113.844.960 m3** in the Ypacarai Hydrographic basin and thus an average yearly discharge of **3,61 m3 /s**.

1: wet year

Table 5-3 Runoff coefficients wet year

Period	month s	rainfall (mm)	Runoff Coefficient	Area (km2) subbasin Yuquyry	Volume of water (m3)
dry	5	75	0,10	302	11.347.573
wet	7	225	0,16	302	76.255.693
				TOTAL	**87.603.266**

A yearly volume in a relatively wet year of **87.603.266 m3** for the Yuquyry stream means an average discharge of **2,81 m3 /s**. and:
This would roughly indicate the following for the entire Ypacarai basin: A yearly volume of **239.542.270 m3** in the Ypacarai Hydrographic basin and thus an average yearly discharge of **7,68 m3 /s**.

This data we used to calculate the estimated quantity of PoCo to be used. For maintaining the lake clean the doses to be applied is different. The average ratio of PoCo to clean wastewater is 1 liter PoCo : 1.000 m3 of water With an average water volume of 64.378.000m3. The total quantity of PoCo will be approximately 64.378 liters (year 1) with an approximate cost of € 4.506.460.

For the second year the quantity will be reduced with
50%, the third year with 40%, the fourth year with 30% and so on until a minimum doses is needed for regular maintenance. It is important that waste water from industries and sewer wastewater is treated as soon as possible. SEAM should propose regulations and sanctions for companies that are not implementing the measures.
See chapter 7.1 for detailed CAPEX and OPEX calculations.

REMARKS and CONCLUSIONS by WiseUse on above stated study and results
- Not all the water that enters the wetlands discharges really into the lake.

- The infiltration and evaporation of the smaller (eastern and western) sub basins may reduce substantially the net discharge to the lake.

- The linear correlation between the Yuquyry sub basin and the other sub basins of the Ypacarai Basin has its limitations (will not be linear) The dendritic pattern of the Pirayu stream and tributaries is quite different than that of the Yuquyry stream; the hydric contribution to the lake will be less than theoretically adopted in the study.

- During periods of heavy rainfall the water levels in the wetlands may temporarily be high without a direct discharge to the lake.

- The evaporation downstream of the discharge measurement stations (in the wetlands before entering the lake) may be high during the year, up to more than 10% of the recorded flow volume.

- The geometry of the Ypacarai Basin may slow down the inflow of the rain water (the hydrograms). Especially during the more dry period the (not saturated) subsoil is more suitable for infiltration.

- The calculation of the discharge of the Pirayu stream into the lake by correlating with calculated factors form the Yuquyry stream (thus without in situ measurements) may provide figures that are far too high. The differences in geomorphology between both sub basins is substantial (runoff patterns, slope and subsoil).

5.2 LOBBY WITH KEY DECISION MAKERS TO ENSURE ITS REALIZATION
Proposal will be presented to MOPC, the study's main counterpart. The ministries have to include all projects and expenses in their annual budget proposal which is then approved by the a technical committee. This committee uses external consultants and other ministries to assess priorities and proposed actions. If projects are approved it will then be decided how its financing will take place.

The project is dependent on priorities. Cleaning the lake has a high priority. Paraguay intends to enhance the tourism industry and the lake is one of the cornerstones of the 59 tourism industry. President Lugo's new administration has signed a memorandum of understanding to address the lake's recuperation.

Wise Use is in close contact with MOPC. CEMIT, and also with pertinent NGO's such as Consejo de Agua and Fundapueblos. Contacts have also been made with the Interamerican Development Bank (IADB) and a governmental fund for new projects. The first step in this lobbying process is the presentation of this report.

6

ORGANISATIONAL & INSTITUTIONAL OBJECTIVES

6.1

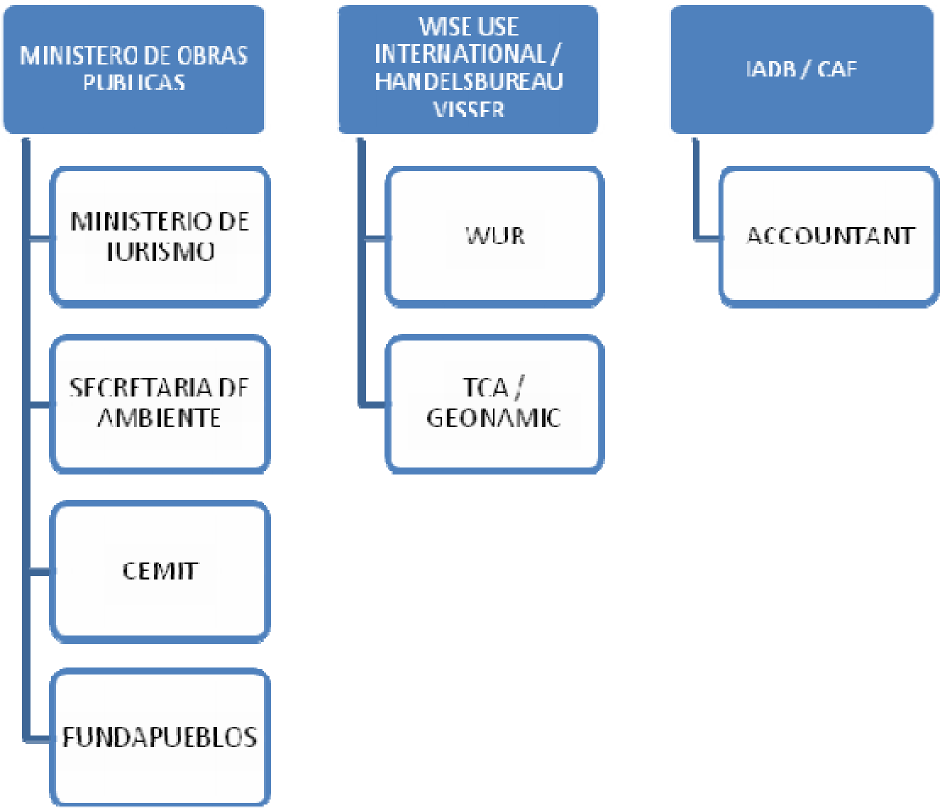
STAKEHOLDERS' ASSESSMENT AND DECISION MAKING PROCESS

6.1.1

STAKEHOLDERS IDENTIFIED

Figure 6-1 Key stakeholders

Figure 6-1 Key stakeholders



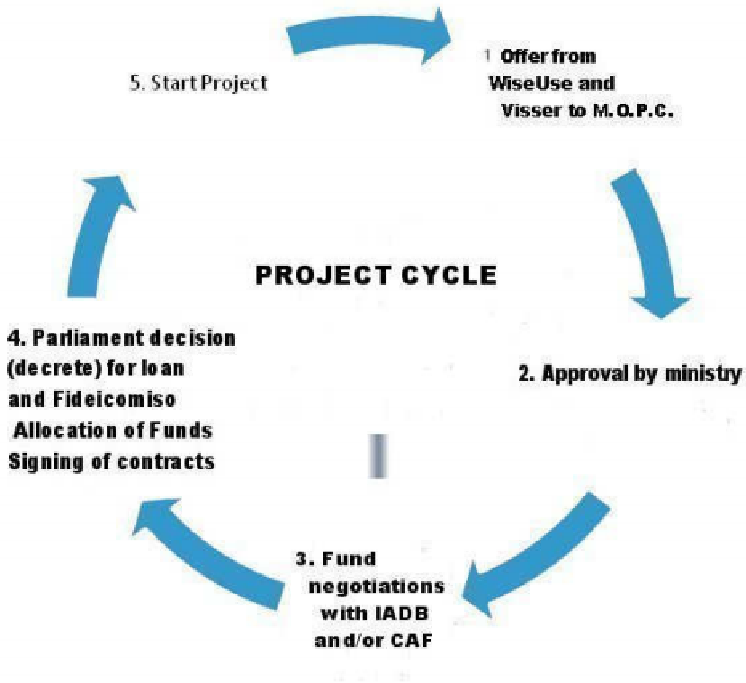
6.1.2 DEFINITION OF KEY RESPONSIBILITIES OF PARTIES INVOLVED

WISE USE / VISSER	WUR / CEMIT	MOPC/SEAM/FUNDAPUEBLOS
•ENGINEERING •TESTS IN SITU •PoCo DELIVERY •DELIVERY OF EQUIPMENT AND MACHINES •INSTALLATION •COMISSIONING •TRANSFER OF TECHNOLOGY •ACCOUNTANCY	•DESIGNING PROTOCOLS FOR THE TESTS •BIO CHEMICAL LAB TESTS OF WATER AND SLUDGE OF THE LAKE •TESTS AND SAMPLING IN SITU	•TECHNICAL AND LOGISTICAL ASSISTENCE IN PARAGUAY DURING THE TESTING PERIOD •PROVIDING OR OBTAINING FINANCIAL RESOURCES (IF OUTCOME STUDY IS POSITIVE) •PROVIDING DATA AND STATISTICS •LOBBYING AT IADB / CAF •LOCAL ACCOUNTANCY

6.1.3 DECISION MAKING PROCESSES AND COMMUNICATION

The decision making process for the commissioning of the work is depicted below. Prior to taking a decision, MOPC will assess the project's feasibility and also other stakeholders will be consulted. Both the IADB and CAF are expected to finance the project through the use of a *Fidei Comiso* which is a sort of escrow account for Wise Use's guarantee.

Figure 6-2 Project cycle



6.2

PROJECT MANAGEMENT STRUCTURE

Whenever the Paraguayan Government decides to invest economically in the cleanup and preventive measures (WWTP with BF) it is a well known and accepted way in South America to use an Trust (Fideicomiso) form. The legal structure of this trust, its trustees, beneficiaries etc should be considered. First talks have already taken place. It is up to the government of Paraguay to decide if the funds, once obtained from financial organizations like the IADB, may be used through a trust. The project management structure, (its executive part) will be headed by a Project manager, in this case appointed with approval of the M.O.P.C. The departments will be: environmental (general applications and existing laws), biochemical department, Engineering, logistics and Financial. Next point is the incorporation via Consulting Committee(s) of NGO's, the Water board (Consejo de Aguas) and the local representatives (mayors) of the municipalities of the Ypacarai Basin as members. These consulting committees are of vital importance for the success of the works.

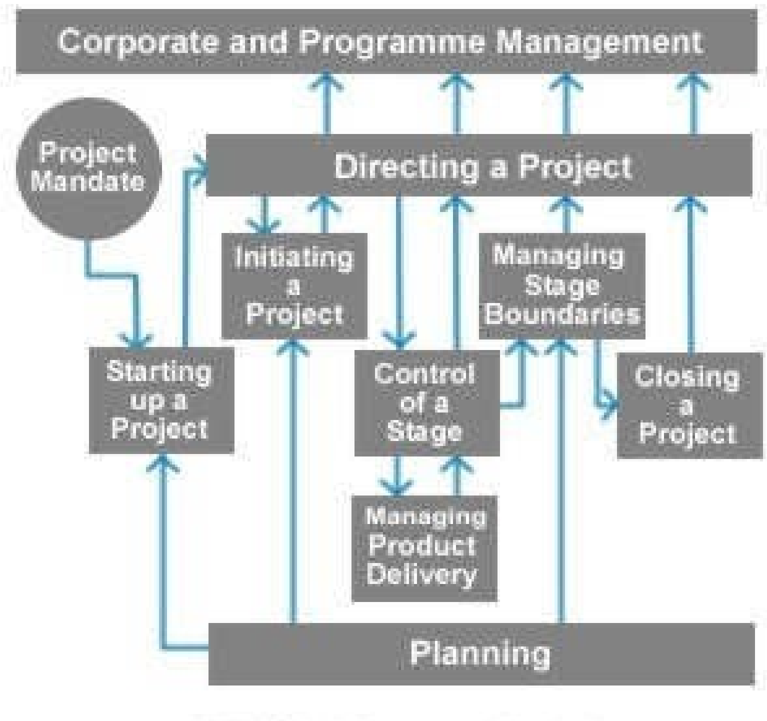
It has no use to go more into detail in this stage of the feasibility study, but some ideas have been proposed.

The new government is highly receptive to using PRINCE2 as project management methodology. This is a major project and Paraguay has had many bad experiences with managing infrastructural projects in the pasts. The additional costs for project management are included in the Wise Use proposals.

Figure 6-3 Project management structure

Fundamental Principles:

1. Trigger: the urgent need for cleaning the Ypacarai Lake.
2. Kick Start: after defining responsibilities of involved parties and final approval
Project Board and financial institutions.
3. Appointing Executive and a Project Manager (Mandate)
4. Designing the Management Team.
5. Preparing a Project Brief with the fundamental principles and context.
6. Defining the Project Approach.
7. Planning and Initiation of the project.
8. Stage controls
9. Managing the Stage boundaries
10. Closing the Project



PRINCE 2 Process Model

7 FINANCIAL OBJECTIVES

7.1 CAPEX AND OPEX

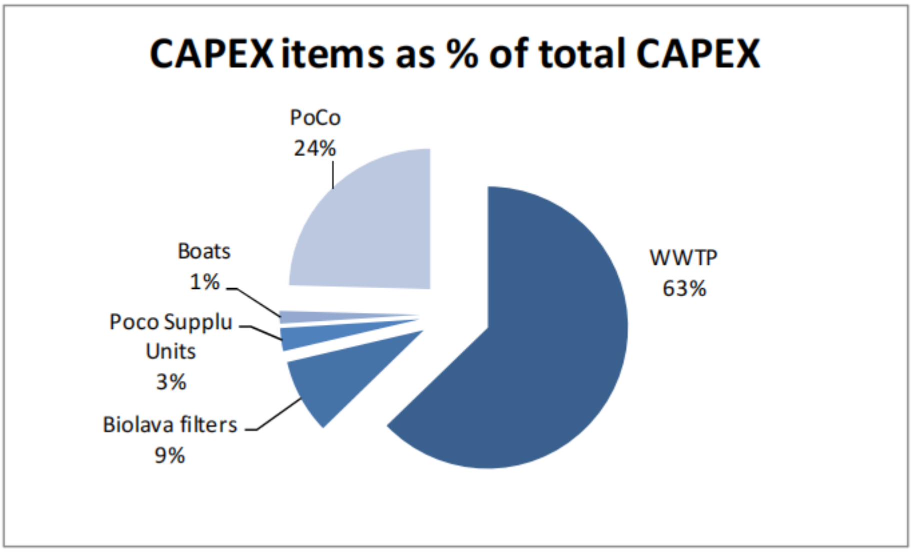
Total CAPEX (Capital Expenditures) in financial prices are presented in the table below. Note that the bulk of these costs are earmarked for Waste Water Treatment Plants (WWTP) to prevent the discharge of untreated water into the lake and thus reversing the Project's cleaning efforts.

Table 7-1 CAPEX in financial prices (Euros and PYG * 1,000)

	No units	costs per unit (euro)	CAPEX euro	CAPEX PYG (*1000)
WWTP	1	21.450.000	21.450.000	135.285.150
Biolava filters	30	98.000	2.940.000	18.542.580
Poco Supply Units	30	30.000	900.000	5.676.300
Boats	1	500.000	500.000	3.153.500
PoCo	120000	70	8.400.000	52.978.800
Total			**34.190.000**	**215.636.330**

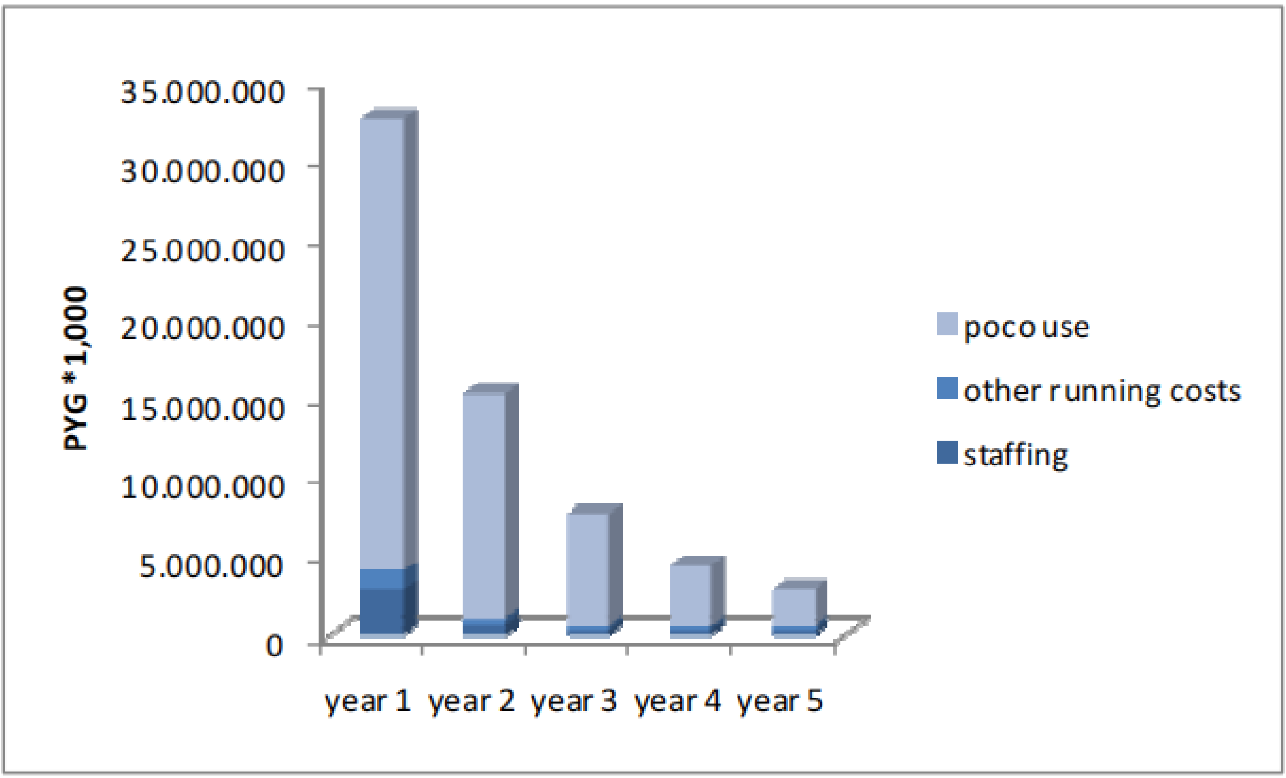
The various CAPEX components from the table above are then presented below as percentages of total CAPEX.

Figure 7-1. CAPEX items in % of total CAPEX



OPEX for the first five operating years are depicted below. OPEX are assumed to remain at the level of operating year five.

Figure 7-2. OPEX (PYG *1,000)



7.2

COST EFFECTIVENESS

The use of an environmental disk cutter dredger costs about USD 4 per m3. Considering that about 30 million m3 will have to be dredged, this entails in a total costs of about USD 120 million, or about 92 million Euros. Compared to the proposed Wise Use solutions this is far more costlier. In addition, the Wise Use solution includes a WWTP which the dredging does not accommodate.

8

ECONOMIC OBJECTIVES

The economic analysis aims on assessing the Project's impact on the region's economic development and/or the country as a whole. The Project's economic feasibility is assessed using two metrics: (1) the Economic Internal Rate of Return (EIRR); and (2) the Net Present Value of the Project's cash in – and outflows. The Project's cash flows will be determined using the "with project" scenario against the "without project" scenario. The "with project" option is represented by the implementation of the proposed priority investment programme whereas the "without project" scenario is the existing situation. Other factors such as the environmental and social impact of the proposed project are also taken into consideration in assessing its economic viability. However, it is stressed that many of these benefits and costs cannot be readily quantified in monetary terms.

A project may be defined as: “the smallest productive entity, physically and technically integrated, that fully utilises the proposed investment and captures all financial benefits that can be attributed to the investment”. **The Project is defined as the biochemical cleaning of the Ypacarai lake, Paraguay.**

8.1 MAIN ASSUMPTIONS USED

The Project’s financial data are already provided in the previous section and these are used and converted into economic values, if necessary. The main assumptions used in the economic analysis are presented in the section below.

Discount rate

The Project on hand is fundamental for social, environmental and tourism development purposes. The opportunity cost of capital used in the economic analysis therefore takes an appropriate rate for the provision of social infrastructure, i.e. 5% as suggested by the EC (Guide Cost-Benefit Analysis Investment Projects). The rate takes into account the economic welfare of the proposed investment for the provision of essential economic infrastructure that primarily produces environmental, health and social welfare benefits.

Conversion Factor

Approximately 20% of CAPEX and OPEX is of local origin. For these local costs, the Standard Conversion Factor (SCF) will be used to convert the financial prices into economic values. Standard Conversion Factor (SCF) calculation for Paraguay ensues in 0.94, included in the annexes.

Exchange rate

The exchange rate applied is PYG : EURO = 6,307 PYG: 1 Euro.

Project evaluation period

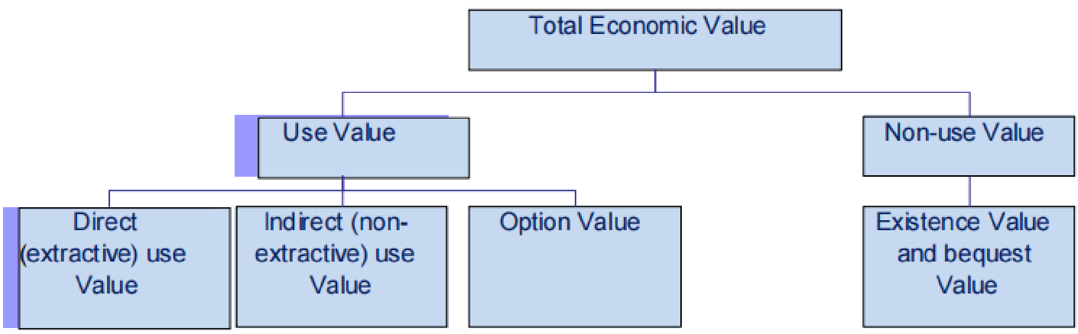
The Project’s evaluation period is 20 years, with 2009 used for the Project’s implementation; its operating period starts in 2010.

8.2 ECONOMIC BENEFITS

The Project’s economic benefits can be divided into two main categories, namely benefits related to use value and benefits related to non-use value.

8-2 Components of economic value

8-2 Components of economic value[5]



The different components of the economic value are further explained in the box below.

Box 1 Economic value components
Direct use value.
Direct use value, also known as extractive, consumptive, or structural use value, derives from goods which can be extracted, consumed, or directly enjoyed. In addition to directly consumed goods, direct use values can also be non-consumptive. All of these benefits are real, can be measured, and have values. Consumptive use is generally the easiest to value, since it usually involves observable quantities of products whose prices can usually also be observed. Non-consumptive use is often more difficult to value since both quantities and prices may not be observed.

Indirect use value.
Indirect use value, also known as non-extractive use value or functional value, derives from the services the environment provides. For example, wetlands often filter water, improving water quality for downstream users, and national parks provide opportunities for recreation. Measuring indirect use value is often considerably more difficult than measuring direct use value. The "quantities" of the service being provided are often hard to measure. Moreover,
many of these services often do not enter markets at all, so that their "price" is also extremely difficult to establish. For the current project, the indirect use value could be the health and environmental effects of waste being collected.

Option value.
Option value is the value obtained from maintaining the option of taking advantage of something's use value (whether extractive or non-extractive) at a later date. It is, therefore, a special case of use value, akin to an insurance policy.

Non use value (existence and bequest value).
In contrast to use value, non-use value derives from the benefits the environment may provide which do not involve using it in any way, whether directly or indirectly. In many cases, the most important such benefit is existence value: the value that people derive from the knowledge that something exists, even if they never plan to use it. Bequest value is the value derived from the desire to pass on values to future generations. Nonuse value is the most difficult type of value to estimate, since in most cases it is:

World bank, Economic Analysis and Environmental Assessment, Environment Assessment Source Book – Update, Number 23, April 1998
and bequest
Value
not, by definition, reflected in people's behavior and is thus wholly unobservable.
(World Bank, 1998)
For the current Project, the main economic benefits related to use and non-use value are presented in the table below.

Table 8-1 Economic benefits

Table 8-1 Economic benefits

Categories of economic benefits	Identified benefits
Use values	
Direct benefits	- None
Indirect benefits	- Benefits for tourism, health and the environment
Option value	- Having a clean environment in the Ypacarai lake vicinity, preserving its use for tourism, residential purposes and other uses
Non-use values	
Non use value	- The knowledge of currently having a clean environment in Ypacarai lake - The certainty of having a clean environment in Ypacarai lake for future generations

8.3 ASSESSMENT OF ECONOMIC COSTS OF THE PROJECT

Both financial values for CAPEX and OPEX from the preceding section are transformed into economic values using the Standard Conversion Factor. All calculations and cash flow tables are included in the annexes.

Table 8-28. CAPEX in economic prices

Table 8-28. CAPEX in economic prices

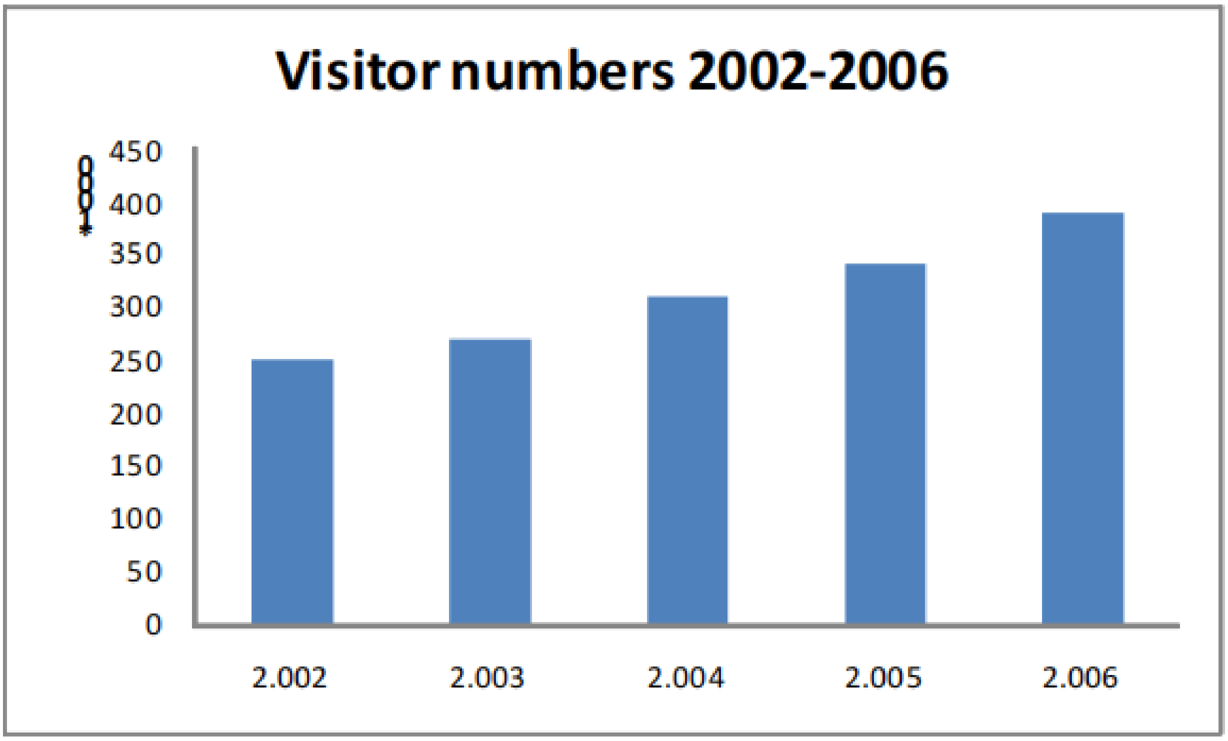
	CAPEX Euros	CAPEX PYG (*1000)
WWTP	20.163.000	127.168.041
Biolava filters	2.763.600	17.430.025
Poco Supplu Units	846.000	5.335.722
Boats	470.000	2.964.290
PoCo	7.896.000	49.800.072
total	32.138.600	202.698.150

8.4 ASSESSMENT OF ECONOMIC BENEFITS

The following economic benefits can be dissected and these are further quantified in the annexes.

1. Tourism
Tourism to the lake Ypacarai is likely to increase if the lake has been cleaned and offers modern tourism facilities, such as hotels, restaurants and water sports facilities. Economic benefits emanating from tourism are, for instance, tourism spending at hotels, bars and restaurants, excursions and any other money spend while visiting the lake. Information on the actual number of visitors arriving in Paraguay is presented in the figure below.

Figure 8-1 Visitor numbers arriving Paraguay



2. Employment generation
Employment generation is linked to tourism activities at the lake. Increased tourism activities entails in a larger number of staff needed to meet tourists' demands for services. In an economic analysis it might be debatable to include the salaries of incremental employment as an economic benefit as this depends on unemployment and rivalling opportunities of staff. Therefore, this report only includes as an economic benefit the salary taxes (accruing to the Government) and a conservative multiplier effect of the augmented employment.

3. Real estate price increases
Real estate (housing and land) in the lake's vicinity are likely to increase in value after the clean up. Augmented economic activities and the attractiveness of the lake shores for second houses will embolden demand for existing houses and drive construction for new ones. Linked to this, the land value is also likely to increase.

8.5 ECONOMIC METRICS
It is stressed that the economic analysis is carried out with the limited information made available and its results can be considered as indications only. Nevertheless, its strong economic metrics suggest that the Project is both viable and recommendable from an economic perspective.

The limited CAPEX, in conjunction with a strong impact on the lake's population, the house stock's value augmentation and generated employment, result in robust economic 69 metrics. As a benchmark, the Project's EIRR should be compared with its benchmark or cut-off rate of 5%, that is the rate the EU uses as discount rate for environmental and infrastructure investments ("EU Cost-Benefit Analysis Guidelines", including the modifications EC 2002, and 2003)6 . This implies that the Project's economic metrics far exceeds the benchmark imposed by the EU.

Figure 8-2 Economic metrics

Figure 8-2 Economic metrics

EIRR (%) (before financing /taxes)	38%
NPV (PYG * 1,000)	358.106.788
Pay Back Period (years)	3

9 LEGAL OBJECTIVES

9.1 PREPARATION OF PRELIMINARY CONTRACTS

An example of an envisaged contract is included in the Annexes.

10 *ENVIRONMENTAL OBJECTIVES*

Introduction
For many decades in Paraguay and especially in the built up areas the environmental awareness was not a major priority with the population nor the government. But after decades of dumping in the Ypacarai lake, the largest and most important in Paraguay, one realized that this harmed the environment but in the first place the inhabitants themselves. Many touristic attractions, like hotels, yacht clubs, private weekend houses were closed, or less visited. This awareness leaded to the founding of "Fundapueblos" a foundation whose main objective is to capacitate (or make aware) the population and local authorities on the consequences of this unrestrained dumping.
The environmental setting of the Ypacarai Hydrographic basin.
As described above in chapter 4 the Ypacarai basin receives, mainly through the Yuquyry subbasin, quite a lot of contaminated wastewater. This has happened for decades now and the buffer function of both wetlands and lake seems to be diminished quite a lot. The black (sometimes sticky) bottom layer, which may locally have a thickness up to 1 m and the algal bloomings which frequently takes place witness the eutrophic to hypertrophic state of the lake. Although rainfall in in theory enough to renew all the water of the lake in

a relatively short time (some years), this is not enough. The lake bottom contamination will not disappear by natural means in reasonable time (a few years).
Limiting the contaminated industrial wastewater will have a direct effect, but the black bottom sludge will stay and this needs to be removed or by dredging or by biochemical means (biocatalyst). Tourist and residents in the region hope that once the lake will be the "Lago Azul". This of course is a mythe as the natural lake bottom is silty and there will always be sediments in suspension. The "blue lake" will be blue by reflecting the blue skies at sunset. The existences of the wetlands both at the entrance and the outlet side of the lake have an enormous protecting capacity against organic contamination, but if the supply of contaminated water is too big the ability to perform this phyto-purification decreases. This is now the case within the Yuquyry sub basin. Therefore a possible solution has been pursued in this study to both protect and sanitise the lake and wetlands. The preceding chapters have indicated how this problem may be tackled. Important from the Jica and UNA studies is that apparently not the domestic wastewater is the source of the greatest contamination nor the tanning industry but all "mataderos" (slaughterhouses) that mostly have no aeration- and sedimentation ponds and discharge freely on the Yuquyry stream or its tributaries. This does not mean that domestic wastewater should not be controlled at the source. The tourist industry is yet not fully developed, and if the lake and incoming waters will not be treated it will not develop any further. The paradox is now that contamination by the tourist industry itself is small, but will be growing whenever the sanitation is performed, and thus will create its own environmental problems if not well tackled . Nature itself created the sanitation buffer zones of the lake. It is the goal of Fundapueblos to simulate public awareness of this vital environmental issue of the Ypacarai Lake and promote measures to be taken by pointing this out at local and state authorities responsibilities. The existence of environmental laws can help but not if the framework is not thoroughly founded.

10.1 ASSESSMENT RELEVANT LEGAL FRAMEWORK FOR EXECUTION OF THE WORKS

1. Two important Laws are governing the environmental issues in Paraguay:
1: LEY N° *369/72* "El servicio nacional de saneamiento ambiental SENASA" and
2: LEY N° *836/80,* el Codigo Sanitario.
Extracts from both laws are included in the annexes. But as with all laws, the regulation on how to execute and control is the most important In Paraguay the Secretariat of SEAM (Secretariat of Environment) depends straight on the Presidency. This means also that regulations are multi ministerial. The first step in the case of the Ypacarai hydrographic basin is the installation of a Water Council (Consejo de Aguas). This is relatively recent and preparation and advise to the SEAM is one of the tasks they should perform and are discussing now. Generally speaking the regulations of the laws (by decrees) and their control and penalty policies have yet to be made. It might well be that once the Lake is recuperated and the polluting industry has sufficient treatment plants installed that this boost (together with greater pollution awareness) stimulates a fair and feasible environmental regulation.

10.2 DEFICIENCIES IN THE ENVIRONMENTAL LAWS

As stated above, the existing environmental laws are sufficient to protect the environment from pollution. But the judicial regulations and their framework is not yet well defined. As some tanning industries discharge their effluent into the Yapacarai Basin waters, it could well be that someday the heavy polluting chromium will (by law) be replaced by other means (enzymes for instance).

11 *RESULTS OF THE STUDY AND CONCLUSIONS*

Results:

The PESP study was very well received by the MOPC, SEAM and other organizations such as FUNDAPUEBLOS and Consejo de Agua. They are trying for a long time to find a integrated solution for the clean up of the polluted Ypacarai Lake and have carried out many investigations to determine what to do. Possible solutions where named: dredging the lake or connect the towns around the lake to a sewer system. SEAM has prepared a package of new laws but still there was no consensus possible with local industries and mayors of the 22 towns around the lake. Fundapueblos started in 2006 and 2007 a campaign to train local political heavyweights in pollution prevention. With a lot of media attention this initiative was a big success and people started to understand the message: “when we keep polluting our lake, the lake will die and Paraguay’s “blue lake” will be lost ”. Recently, a month before the presidential elections of 2008, Fundapueblos also achieved that all presidential candidates compromised themselves to give high priority to a quick action plan in order to start cleaning up the lake and start the prevention of polluting it further. The temporary laboratory investigations of CEMIT and Wise Use where presented and the promising results gave hope to the ministry. We can conclude that the tests with PoCo have accelerated the interest of the community and local politics. There is new hope that PoCo shall contribute to clean up the lake. The lab reports of CEMIT University where very promising and convincing. The Inter American Development Bank (IADB) is visited by a delegation of MOPC and Wise Use. They are very interested to study the case and start negotiations with MOPC as soon as the results are official.

Conclusions:

1. The Ypacarai Lake is highly polluted with mainly organic waste proceeding from non treated domestic wastewater and industrial loads. It is not safe for recreation purposes due to high concentrations of Cyanobacteria and Coliforms.

2. Until a few years ago there was little or no (political) interest in cleaning the Lake. Mainly due to the lack of viable solutions and frustration. However up to day the bureaucracy of the former decade is reduced and the ministries (e.g. MOPC and SEAM) have a close working relation. The environmental laws are ready now for regulation input, followed by implementation.

3. Private initiatives developed by Fundapueblos proved to be a good advisory staff for the ministries. The Consejo de Agua (Water Council for, for instance the Yapacrai Hydrographic Basin) is inter-institutionally still not well defined. Public and Private sector are really working together now to find a integrated solution for cleaning up the lake.

4. The possibility to dredge the lake was far too expensive (over US$120 million) and does not lead to a sustainable solution for a clean lake.

5. The possibility for the implementation of 22 wastewater treatment plants, carried out by MOPC, is still open. The solution that Wise Use provides (WWTP and Biological Filters i.e. BF for the Operational Industries) in Yucuyry and bio filters directly at the industries) also is viable. It might well be that a combination of both solutions will give the best results.

6. The product PoCo has been tested in laboratories and in upgrade scale in situ (Nautic Club in San Bernandino) with very promising results. Important issues, such as reduction of Cyano and Coliform bacteria have been achieved in very short time (within 5 days) to acceptable levels for save water recreation. (micro bacteriological) These results are scientifically backed up. (see attached report of CEMIT)

7. The implementation of PoCo in WWPT and BF (bio-filtering) is the first step to an integral solution for cleaning up the lake. It is also far cheaper than other solutions offered.

8. PoCo shall be fabricated in Paraguay since transporting a ready to use product with high content of water will be too expensive. Wise Use provides the basic ingredients and invests in a local factory.

9. Local content will be very high in this project. Besides the fact that PoCo will be produced locally, the WWTP and parts of the BF will be constructed and engineered by local companies, using local products. The role of the Dutch companies will be limited to delivery of some machines, instruments and supervising.

10. When the Lake is in process of being cleaned, international and local tourism will boost. New investments will be done in hotels, restaurants, Recreational Clubs and infrastructure in order to receive more tourists.

11. The Project's economic metrics point to its strong value creation in relation to a limited CAPEX and OPEX. Its EIRR far exceeds the benchmark imposed by the EU and it can be concluded that the Project is both viable and recommendable from an economic perspective.

Research Water Pollution Control Bolivarian Republic of Venezuela 2013

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Study on the effects of PoCo (Pollution Control) on the sustainable sanitation of Lake Maracaibo and its impact on crude oil leaks

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Saint Rita, May of 2013

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Ministerio del Poder Popular para la Alimentación

PDVSA

SUMMARY

The objective of this study was to determine the effectiveness of the PoCo (Pollution Control) product for the cleanup of Lake Maracaibo. The first part of the tests was based on determining small-scale technical feasibility. The second part, yet to be approved and implemented, involves conducting a full-scale test. It should be noted that this type of test is representative of the current situation in the lake, since on a small scale (tanks filled with 700 liters of Lake Maracaibo water each) there is no presence of flora, fauna, marine currents, temperature, mud, lake sediments, etc. The purpose of this first test was to observe some of the reactions when 1 ml of crude oil from Lake Maracaibo was placed in the tank.

The test was carried out jointly with the Venezuelan company, Petróleos de Venezuela (PDVSA), with a shorter protocol, with constant mechanical agitation, where at the end of the test and **IT CAN BE OBSERVED THE REDUCTION OF THE RAW OF EQUAL SHAPE** , but HE
It is recommended that, in order to complete the reaction of the microorganisms, a further study of the effects of PoCo on stable emulsions of hydrocarbons with water should be carried out over a longer period of time.

The study provides extensive information on the test protocol and results. However, **we have visually observed the start of a reaction in the tanks almost immediately after adding the little bit, such as: crude oil separated from the tank surface, formation OF BUBBLES, SEPARATION OF WATER AND OIL, FORMATION OF SEAWEED, SMELL AND MUD. TO THE THIRD DAY HE**

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HE CLEARLY NOTICED A DECREASE (BETWEEN 40-50%) IN THE AMOUNT OF SUSPENDED OIL IN THE WATER IN THOSE TANKS WITH A LITTLE DOSAGE, WHERE THE ONE WITH THE HIGHEST DOSAGE DEMONSTRATED AT A VISUAL LEVEL SHOWED A FASTER SPONTANEOUS REACTION DESPITE THE FACT THAT THE WATER PRESENTED GREATER TURBIDITY.

PARTICIPANTS

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INTRODUCTION

Wise USE International BV, a company devoted to developing and Produce biological products based on plant extracts and fruit oils, which are capable of activating and accelerating the metabolism of microorganisms under aerobic or anaerobic conditions. He developed a biocatalyst called Pollution Control (Bit), 100 % natural and Biodegradable. PoCo can be used to treat municipal and industrial effluents and neutralize the toxic effects of oil spills on the environment. The product restores the natural balance of the water by activating the lake's natural microorganisms, which have been dormant and suppressed by pollution.

The purpose of PoCo is to stimulate and accelerate the growth of "good" microorganisms in a natural and accelerated manner. An accelerated metabolism results in a high rate of decomposition, demonstrated by increased production of natural gas (methane), CO2 . and N_2 , rapid decomposition, better transfer of Ammonium, water clarity, odor reduction, etc. In the case of oil leaks, the little activates certain colonies of latent microorganisms in the water that accelerate the decomposition of floating and emulsion oil, through a catalytic reaction.

In he year 2007 the signatures Wise USE International BV, and formed by Dutch consultants: Wageningen University's Centre for Water and Climate; the Aquatic Ecology and Water Quality Management group, Eng. Laurens Trebes and Manuel Perez (TCA Consult), Dr. Ir. Marinus Pool (Geonamic), and Dr. Ronny Venegas Carbonell. Together with the Ministry of Public Works and Communications (MOPC), the Environment Secretariat, and the Ministry of Industry and Commerce, they conducted a feasibility study to biochemically treat he Lake Ypacaraí in Paraguay he which was highly

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polluted simply by the uncontrolled discharge of industrial waste and sewage.

This study consisted of several stages, a first pre-PESP study where a series of preliminary pilot tests were carried out in March 2007 in the laboratories of the National University of Asunción, Faculty of Biochemistry (CEMIT) by a Wise Use biochemist in cooperation with the staff of the University, in presence of several Engineers and of the Ministers in attendance. The purpose of this pilot study was to confirm the effectiveness of PoCo as a biocatalyst to reduce the level of contaminants in Lake Ypacaraí. Test samples were contained in three 500-L plastic tanks, designed by loading, where water was pumped from the bottom and middle of the lake, as these are highly contaminated areas where sticky black bottom sediments were present. The procedure shown in Table 1;

Name	Samples	Grades
Tank Yo	500L sample + aeration	
Tank II	500L sample + aeration + Bit @ 10 mg/L	He Bit was added @ 0 h
Tank III	500L sample + no aeration + PoCo @ 10 mg/L) maintaining an anaerobic state + agitation Manual by 5 min @ intervals of 2 hours	He Bit was added after 36 h in condition anaerobic

Board 1. Content of the tanks during he rehearsal pilot .

These experiments continued for 5 days, followed by continuous aeration for tanks I and II, while tank III was agitated manually only. Samples were analyzed for 0 hours, 48 hours, and 120 hours. The results were as follows: COD, coliform, and solids levels decreased considerably. This behavior corresponds to to the activity of life mycobacterial that consumed all the possible nutrients in that specific amount of water. We also observed a reduction in the odor and color of the water, which became clearer over time.

An on-site test was subsequently conducted to obtain more reliable data. The product dosage was 1 liter of PoCo per 1,000 m ³ of water. lake. The evidence began he 11 of March of 2008 until he 16 of May 2008 at the San Bernardino Nautical Club, inside a closed lake pool of approximately 40 x 40 meters and 2 meters of depth, with a volume of about 3000 m ³ . The thickness of the biological layer on the bottom (sludge) was measured at about 40 cm, and was similar to that of the lake. After the introduction of PoCo, samples were taken daily from March 12 to 23, and then weekly from March 25 to May 7. About 100 grams of biocatalyst was added daily from March 12 to 18, then 200 grams from March 19 to 25, and again 100 grams from April 2 to 15, and finally, 200 grams from April 16 to 20 and from April 21 to May 7. Analyses were based on Standard Methods for the Analysis of Drinking Water and Wastewater (*Standard Methods, 20th Ed*), for physical, chemical and bacteriological parameters.

The results obtained were: change from opaque water to a slightly more transparent one when the PoCo dosage began, followed by an increase due to microbial activity in the sediments resulting in the re-suspension. The Chemical Oxygen Demand (COD) did not show significant variations at all stages of the test. Total phosphorus increased; this may be due to the increase in microorganisms that degrade organic material, and therefore release phosphorus in organic form, and also due to the change from acidic to alkaline pH. A decrease in total coliforms and cyanobacteria was also obtained with the addition of the little ; after 5 days, the same decrease was shown, IT WHICH IS OF GREAT IMPORTANCE, POSITION THAT

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ONE OF THE MOST SERIOUS PROBLEMS OF LAKE YPACARAÍ IS THE DEGREE OF EUTROPHICATION.

The first goal of cleaning the Ypacaraí is to make it safe for recreation. According to the results of the second test, it was observed that the water quality had improved considerably from Category 5 (recreation not permitted) to Category 2 (safe for recreation), resulting in the implementation of little in the treatment plants. OF WATERS RESIDUALS AND OF BIOFILTERS OF LITTER
FIJOS is the first step in a comprehensive solution for cleaning the lake and is also more economical than other solutions offered to the Paraguayan government.

Lake Maracaibo is located in western Venezuela, in the state Zulia. Its surface area actually corresponds to the lagoon largest in the world. It has about 13,820 km², making it the lake largest in South America and Latin America. Due to its size, it is considered an inland sea and is ranked as the number 19 among the largest lakes in the world.

Numerous oil leaks, at least partly attributable to poor maintenance of the operations and the indiscriminate discharge of untreated wastewater, have significantly deteriorated the quality of the water, to the point that in the area of Zulia catches have fallen to levels that prevent its exploitation, and in some places the water presents levels of contamination that are very dangerous for health. According to experts, including those from the organization ACLAMA, Lake Maracaibo requires around ten wastewater treatment plants, but apparently there are only two in the region. On the shores of the lake, you can see the advanced stage of propagation of a new green stain that fishermen and residents call *"verdigris"* . To the seem HE treats of Algae green blues (cyanophytes) and the calls *"scum"* can become toxic. These blue-green algae find their best *"food"* in the nutrients provided mainly by wastewater and fertilizer residues, all rich in nitrogen and phosphorus. The Lake itself shows signs of its condition: its capacity to process organic matter (eutrophication) has been exhausted, and the 600,000 liters of rainwater, wastewater, and industrial waste that fall daily have altered the its original chemical composition.

The cleanup of Lake Maracaibo translates into a better quality of life for the entire region. The Ministry of the Environment's goal is to offer the people of Zulia a healthy environment, free of unpleasant odors, a body of water that offers benefits rather than further hardships.

For this reason, the following test consisted of evaluating the efficiency of the treatment of the water of Lake Maracaibo on a pilot scale with the Pollution Control catalyst with the oil spill test, thanks to the positive results previously achieved by the company Wise Use treating with PoCo Lake Ypacaraí to reduce the level of pollutants in the lake.

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Methodology Used.

This chapter presents the procedures, materials, and equipment necessary to carry out this research in a clear, simple, and organized manner, so that the information expressed therein can be understood, allowing the achievement of the stated objectives.

Procedure experimental. Equipment

description.

Six potable water tanks with a useful volume of 700 L were used, see Figure 1, filled using a 1/2 HP BRINK pump with water from Lake Maracaibo from the banks surrounding the pier at the back of the USPS La Camaronera, Santa Rita Municipality, Zulia State. They were in turn equipped with an agitation system coupled to two 1/2 HP three-phase motors that provided the mixture of Pollution Control, Lake Maracaibo water and TJM Medium Crude three times a day.

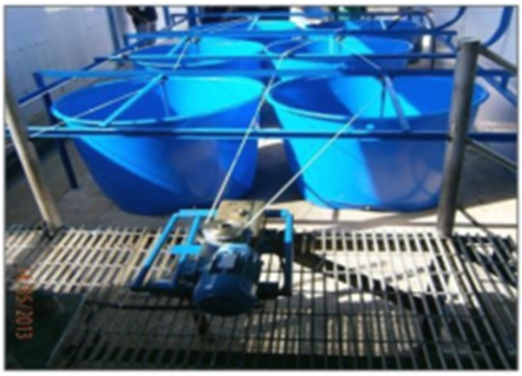

Figure 1. Tanks used in he Essay. Source: USPS La Camaronera

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Rehearsal.

This test used an organic liquid catalyst called Pollution Control (PoCo) observed in Figure 2, produced by the company WISE USE International BV, Lake Maracaibo Water (ALM) and TJM Medium Crude (CM) provided by the company Petróleos de Venezuela (PDVSA). Which was divided into stages shown in Table 1, described below; the first stage from day 2 to day 6 were added to the tanks of the previous stage 2 ml of Bit (tank 2, tank 5), 4 ml of Bit (tank 3, tank 6), and
only 100 ml of CM to 3 tanks on day 2 (tank 4, tank 5 and tank 6).

Tank	Name	Stage 1	Stage 3
1	Pattern Sample	-	-
2	Sample 20 ml	2 ml of PoCo	-
3	Sample 40 ml	4 ml of PoCo	-
4	Pattern Sample	100 ml CM	-
5	Sample 20 ml	2 ml of PoCo +100 ml CM	-
6	Sample 40 ml	4 ml of PoCo +100 ml CM	-

Board 2. Content of the tanks during he rehearsal of sanitation .

Figure 2 . Pollution Control (PoCo).
Source: USPS La Camaronera.

The test was carried out at the facilities of the La Camaronera Socialist Secondary Production Unit

Figure 3. Content of the tanks. Source: USPS La Camaronera.

✓ **First stage.**

The first day was attended by Manuel Pérez, Agroindustrial Coordinator USPS La Camaronera, Mr. Laurens Trebes from the company WISE USE International BV and from the Regional Core 3 Laboratory of the GNB Lt. Marlling Guerra and Lt. Sughaes Sánchez, note in Figure 4.

Figure 4. Staff visitor in the USPS The Shrimp Farm. Source: USPS The Shrimp Farm.

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Initially, Mr. Laurens Trebes, as a representative of the company WISE USE International BV, gave a short training talk about the test that was going to be carried out, what parameters to measure, what were the expected effects with the use of the product as an organic catalyst for the sanitation of Lake Maracaibo and finally provided a summary. from the bibliographic material that dealt with the test they did with the same product in Lake Ypacarai located in Paraguay.

Rehearsal with Bit made with PDVSA

Experiment: following the previous procedure, treat with PoCo two 700L samples of water from Lake Maracaibo impacted with 1L of Medium Crude Oil (MCO) corresponding to a concentration of:

$$[C_1] = \frac{2000000}{700} = 2857\ ppm$$

$$[C_2] = \frac{4000000}{700} = 5714\ ppm$$

Sample Pattern= 700 L water of the Lake from Maracaibo + 1L from CM.
Sample 20 ml = 700L of Lake Maracaibo Water + 1L of CM + 20 ml of Bit.
Sample 40 ml= 700L of Water of the Lake of Maracaibo+ 1L of CM+ 40ml from PoCo.

DATE	VISITS IN THE USPS THE SHRIMP FARM DURING THE REHEARSAL
05/14/2013	Manuel Perez., Coordinator Agroindustrial USPS The Shrimp farm, Laurens Trebes, WISE USE international BV Lt. Marlling War, Laboratory Regional Core 3, Guard National Lt. Sughaes Sanchez, Laboratory Regional Core 3, National guard
05/15/2013	Manuel Perez, Coordinator Agroindustrial USPS The Shrimp farm, PhD. Nolberto Pineapple- PDVSA Gerardo Osorio- PDVSA Laurens Trebes, WISE USE international BV

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	Gianno Chirino, WISE USE- INVATIVE
16/05/2	Manual Perez., MPPA
17/05/2	INZIT
20/05/2	PhD. Nolberto Pineapple- PDVSA Gerard Osorio- PDVSA
21/05/2	Tte. Marlling War, Laboratory Regional Core 3, Guard National Tte. Sughaes Sánchez, Laboratory Regional Core 3, Guard National INZIT
22/05/2	PhD. Nolberto Pineapple- PDVSA Gerardo Osorio- PDVSA

Board 3. Visits in the USPS The Shrimp farm.

Rehearsal with Bit done in set with the PDVSA company

First, the tanks were filled with water samples from Lake Maracaibo, as shown in Figure 5.

Figure 5. Fill of tanks for he rehearsal.
Fountain: USPS The Shrimp farm.

On the first day of the test, hour "0" before adding the PoCo, the samples showed a dark green color, with a sweet fishy smell, very cloudy, as seen in Figure 6.

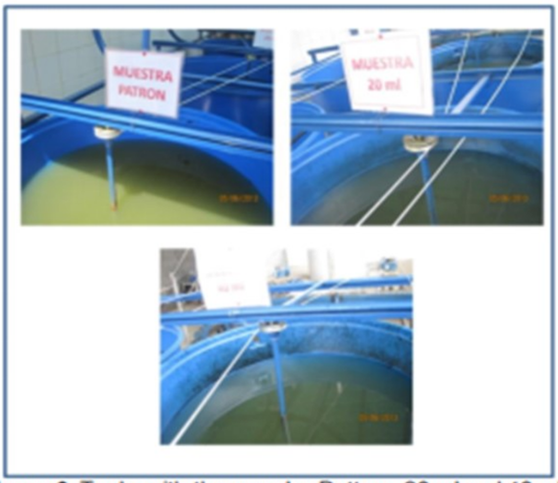

Figure 6. Tanks with the samples Pattern, 20 ml and 40 ml.
Fountain: USPS The Shrimp farm.

This day was the day for sampling by Dr. Nolberto Piña of PDVSA and Attorney Jose Peters of PDVSA, as shown in Figure 7.

Figure 7. Take of samples 1st day of ALM and of ALM+ CM.
Fountain: USPS The Shrimp farm.

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After adding the PoCo 30 minutes in the ALM samples the changes were observed, the 20 ml sample showed a darker green color due to the color of the PoCo (black), the smell of the ALM was the characteristic one distinguished at the beginning of the test, without the presence of algae, very similar to the pattern sample; in the 40 ml sample it was noticed that the water had a darker color than the 20 ml sample, in turn without the presence of algae and the smell was of ALM, by last the sample Pattern remained without changes apparent, As the hours pass, the water in the tanks with the PoCo doses of 20 and 40 ml becomes clearer. See Figure 8 below.

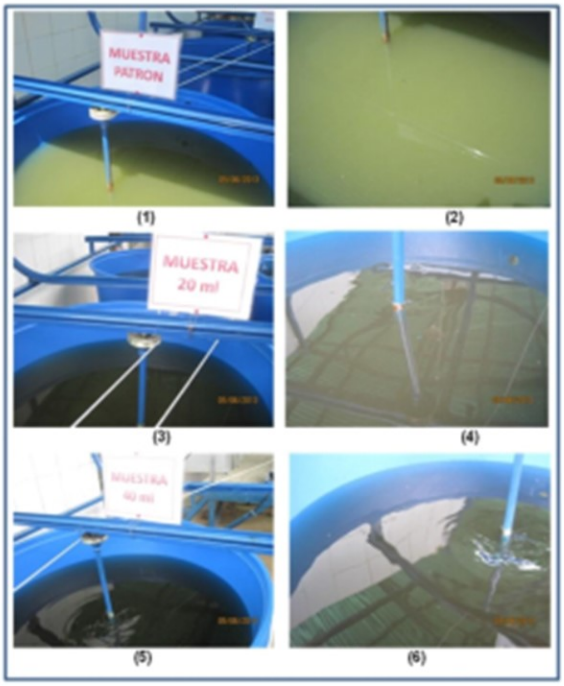

Figure 8. Tanks with the samples Pattern, 20 ml and 40 ml, then of add the PoCo **Fountain:** USPS The Shrimp farm.

Of equal shape in that period of time for the samples of ALM + CM HE visualized small changes see Figure 9, where; the sample of 20 ml

It presented some small traces (lines) on the crude oil, clearly the PoCo was reacting and fragmenting the CM, its smell was characteristic of CM, the presence of algae was also noted on the surface in the form of small particles, thus for the 40 ml sample, the presence of algae was also visualized on the surface of the sample, the CM impregnated the water with its characteristic smell and was noticed fragmented without adhesion on the walls of the tank such as the 20 ml, a very different case to the Pattern sample where the CM remained fluid on the surface, no presence of algae was noticed and the smell was raw in the same way.

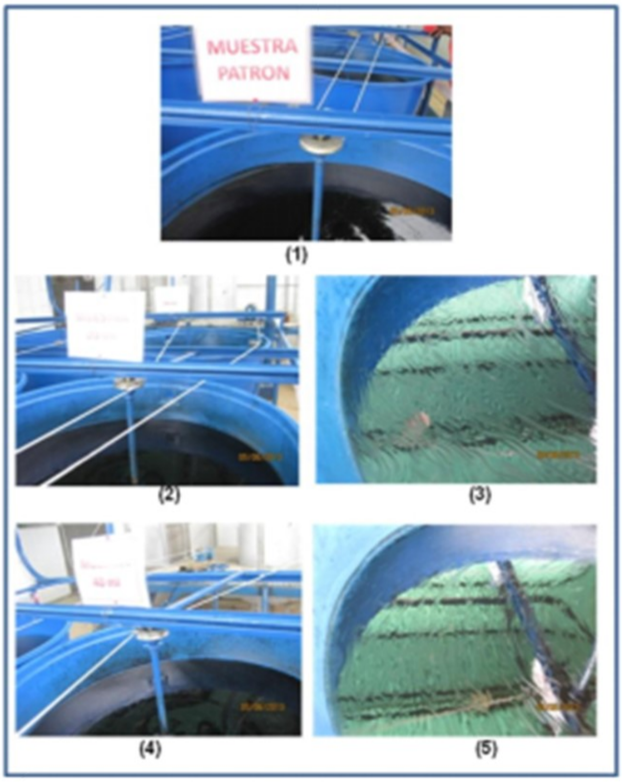

Figure 9. Tanks with the samples of ALM+CM: Pattern, 20 ml and 40 ml, after adding the PoCo
Fountain: USPS The Shrimp farm.

After 24 hours of testing, as shown in Figure 10, changes were observed in the samples: the standard sample had a green color and a characteristic ALM odor, the 20 ml sample had a dark green color, a fishy odor, and small sediments and algae were also observed floating throughout the body of water. The 40 ml sample had a blackish green color, with no fishy odor, and also had algae on the surface, but in smaller quantities than the 20 ml sample.

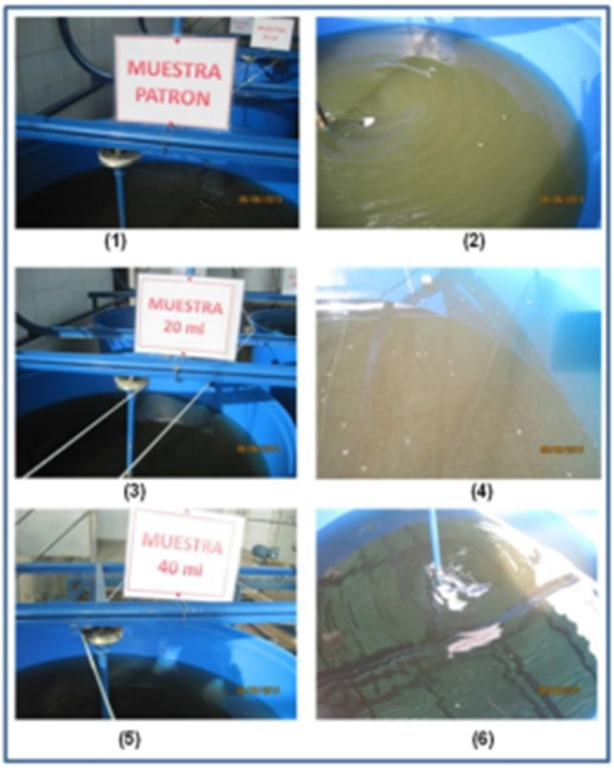

Figure 10. Tanks with the samples of ALM: Pattern, 20 ml and 40 ml @ 24 hours

Fountain: USPS The Shrimp farm.

In the ALM + CM samples it was observed that in the 20 ml sample you could see the ALM with a deep green color, already that the CM was crowded in the center around the stirring mechanism, smell of CM, small accumulations of algae near the tank walls; in the 40 ml sample the CM was dispersed in minimal forms on the water surface, achieving see he ALM contained with a color such to the of the sample of 20 ml, MC odor, there was a small amount of algae in the form of dots; in the standard sample, the MC remained fluid on the surface, immiscible, with bubbles and a characteristic odor. As shown in Figure 11.

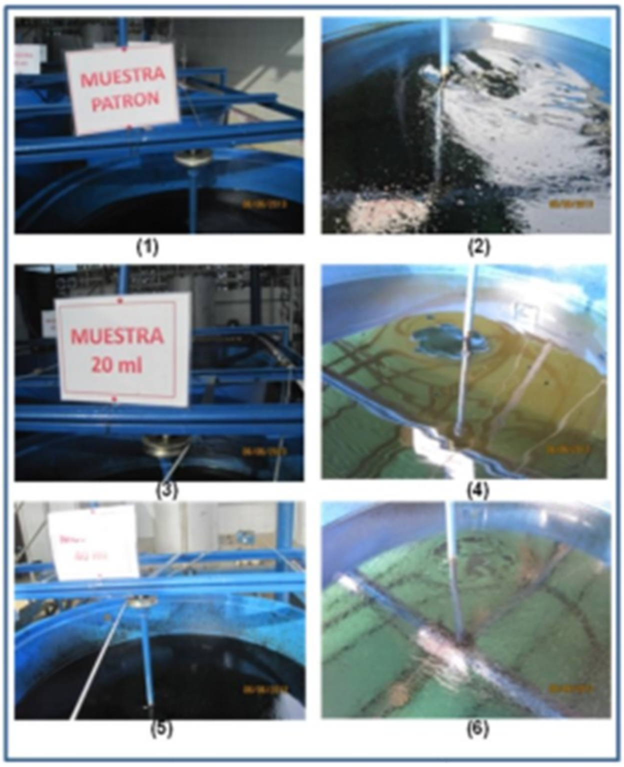

Figure 11. Tanks with ALM+ CM samples: Standard, 20 ml and 40 ml @ 24 hours

Fountain: USPS The Shrimp farm.

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Ministerio del Poder Popular para la Alimentación

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ALMACENAMIENTO TRATAMIENTO Y TRANSPORTE DE CRUDO
TRATAMIENTO Y LABORATORIO
LABORATORIO DE PETROLEO
TIA JUANA

INFORME DE ENSAYO

Solicitado por: MANUEL ANTONIO PEREZ TRUJILLO
Fecha de Toma: 06/06/2013
Fecha de Análisis 06/06/2013

RESULTADO DE ANALISIS FISICOQUÍMICOS AGUA DEL LAGO 06/06/2013

MUESTRA	CONTROL 06/06/13	DOSIS 20ml 06/06/13	DOSIS 40ml 06/06/13
FECHA DE TOMA	06/06/2013	06/06/2013	06/06/2013
FECHA DE ANALISIS	06/06/2013	06/06/2013	06/06/2013
Temp (°F)	78	78	78
pH	8,37	7,85	7,84
ALC.	52,60	51,62	45,13
Na+	778,47	794,18	635,18
Resistividad	3,35	3,38	4,01
Conductividad	NO DETECTADO	NO DETECTADO	NO DETECTADO
CO3=	5,3	NO DETECTADO	NO DETECTADO
HCO3-	58,80	57,60	55,08
Cl-	1415	1398,00	1188,49
Ca++	28,21	25,13	20,40
Mg++	77,00	62,78	75,80
DT	389,60	354,25	354,25
SiO2	13,20	7,94	12,90
SO4=	26,00	24,00	22,00
Fe	N / D	N / D	N / D
Sol. Susp	6,00	6,00	4,00
indice de STIFF 140°F	0,11	-0,44	-0,54
indice de STIFF 180°F	0,45	-0,10	-0,21
indice de STIFF 200°F	0,64	0,09	-0,02
Tendencia de STIFF 140 °F	INCRUSTANTE	NO INCRUSTANTE	NO INCRUSTANTE
Tendencia de STIFF 180 °F	INCRUSTANTE	NO INCRUSTANTE	NO INCRUSTANTE
Tendencia de STIFF 200 °F	INCRUSTANTE	INCRUSTANTE	NO INCRUSTANTE

Realizado por
NESTOR GRANDA

Validado por
NOLBERTO PIÑA LIDER DE LABORATORIO

Revisado por
GERARDO OSORIO

Aprobado por
GERARDO HERNANDEZ GTE. DE LAB. C.O

República Bolivariana de Venezuela
Ministerio del Poder Popular para la Alimentación

PDVSA

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ALMACENAMIENTO TRATAMIENTO Y TRANSPORTE DE CRUDO
TRATAMIENTO Y LABORATORIO
LABORATORIO DE PETROLEO
TIA JUANA

INFORME DE ENSAYO

Solicitado por: MANUEL ANTONIO PEREZ TRUJILLO
Fecha de Toma: 07/06/2013
Fecha de Análisis: 07/06/2013

RESULTADO DE ANALISIS FISICOQUÍMICOS AGUA DEL LAGO 07/06/13

MUESTRA	CONTROL 07/06/13	DOSIS 20ml 07/06/13	DOSIS 40ml 07/06/13
FECHA DE TOMA	07/06/2013	07/06/2013	07/06/2013
FECHA DE ANALISIS	07/06/2013	07/06/2013	07/06/2013
Temp (°F)	78	78	78
pH	8,38	8,32	8,08
ALC.	47,51	43,11	43,73
Na+	877,15	820,79	807,70
Resistividad	3,06	3,26	4,14
Conductividad	NO DETECTADO	NO DETECTADO	NO DETECTADO
CO3=	2,66	NO DETECTADO	NO DETECTADO
HCO3-	55,38	50,01	53,37
Cl-	1564	1488,00	1154,00
Ca++	24,03	21,21	22,94
Mg++	76,00	72,51	75,86
DT	378,60	374,20	373,30
SiO2	9,90	8,23	9,67
SO4=	26,00	22,00	19,00
Fe	N/D	N/D	N/D
Sol. Susp	6,00	22,00	28,00
Indice de STIFF 140°F	0,05	-0,11	-0,28
Indice de STIFF 180°F	0,40	0,23	0,87
Indice de STIFF 200°F	0,59	0,43	0,28
Tendencia de STIFF 140 °F	INCRUSTANTE	NO INCRUSTANTE	NO INCRUSTANTE
Tendencia de STIFF 180 °F	INCRUSTANTE	INCRUSTANTE	INCRUSTANTE
Tendencia de STIFF 200 °F	INCRUSTANTE	INCRUSTANTE	INCRUSTANTE

Realizado por
NESTOR GRANDA

Verificado por
NOLBERTO PIÑA LIDER DE LABORATORIO

Revisado por
GERARDO OSORIO

Aprobado por
GERARDO HERNANDEZ GTE. DE LAB. C.O

NOTA: QUEDA PROHIBIDA LA REPRODUCCION PARCIAL DE ESTE DOCUMENTO SIN LA AUTORIZACION ESCRITA DEL LABORATORIO
FECHA: JULIO 2013

República Bolivariana de Venezuela
Ministerio del Poder Popular para la Alimentación

PDVSA

Conclusions.

- ✓ 1. High efficiency in the degradation of hydrocarbons
  The use of PoCo in aerated systems has demonstrated a remarkable ability to reduce and even eliminate petroleum residue particles, thanks to its specialized microbial action in decomposing hydrocarbon compounds.

- ✓ 2. The aeration power the activity of the bioremediator
  The constant supply of oxygen favors the metabolism of the microorganisms present in PoCo, accelerating the biodegradation of contaminants and increasing the efficiency of the process.

- ✓ 3. Reduction of compounds toxic persistent
  HE ha observed a decrease of fractions complex as Polycyclic aromatic hydrocarbons (PAHs), which tend to be more resistant to degradation, indicating a high potential for PoCo to treat difficult contaminants.

- ✓ 4. Alternative ecological and sustainable
- ✓ Unlike chemical or physical methods, the use of PoCo represents a more environmentally friendly solution, without generating toxic byproducts or significantly altering the aquatic ecosystem.

- ✓ 5. Adaptability to different conditions
- ✓ PoCo has proven to be effective in various vat configurations. and levels of pollution, it that it converts in a

Versatile tool for real-life industrial spill or wastewater scenarios.

- ✓ 6. Conditions Visuals to the results

  Visually, we observed the onset of a reaction in the tanks almost immediately after adding the little bit, such as: crude oil separated from the tank surface, bubble formation, separation of water and oil, algae formation, odor and sludge. A clear decrease (between 40-50%) in the amount of oil suspended in the water was noted in those tanks dosed with PoCo, where the one with the highest dosage visually demonstrated a faster spontaneous reaction even though the water presented elderly turbidity by a process of change in the water.

Recommendations.

- ✓ Determine in more comprehensive tests the presence of heavy metals, petroleum residues and toxic substances in the water throughout the PoCo treatment.

REPÚBLICA BOLIVARIANA DE VENEZUELA
MINISTERIO DEL PODER POPULAR PARA LA DEFENSA
FUERZA ARMADA NACIONAL BOLIVARIANA
GUARDIA NACIONAL BOLIVARIANA
DIRECCION DE OPERACIONES
LABORATORIO CENTRAL
LABORATORIO REGIONAL N°3
JEFATURA

Maracaibo, 20 de Junio del 2013
203° y 154°

No. CG-DO-LC-LR3/1020

DEL : CORONEL JEFE DEL LABORATORIO REGIONAL N°3 DE LA GUARDIA NACIONAL BOLIVARIANA.

AL CDDNO : FELIX OSORIO GUZMAN. MINISTRO DEL PODER POPULAR PARA LA ALIMENTACIÓN.

ASUNTO : REMISION DE DICTAMEN PERICIAL BIOLOGICO Y FISICO-QUÍMICO.

REFERENCIA : OFICIO DE SOLICITUD NRO. 000516 DE FECHA 13MAY13.

Tengo el agrado de dirigirme a usted, en la oportunidad de acusar recibo a la comunicación citada en la referencia, en atención a la solicitud, le remito anexo al presente oficio, el Dictamen Pericial Biológico y Fisico-Quimico número CG-DO-LC-LR3-DQ-13/0914 del 17JUN13, el cual consta de siete (07) folios útiles.

Comunicación y remisión que hago a usted, para su conocimiento y fines legales consiguientes

Dios y Federación,

WAYME JOSÉ MARÍN OCANTO

WJMO/sánchez.-
Anexo: lo indicado...

"CHÁVEZ VIVE... LA LUCHA SIGUE
INDEPENDENCIA Y PATRIA SOCIALISTA... VIVIREMOS Y VENCEREMOS"

Dirección: final de la Av. Goajira con final de la Av. El Milagro, sector las peonias (CORE-3).Correo electrónico: laboratoriocriminalistico3@hotmail.com

REPÚBLICA BOLIVARIANA DE VENEZUELA – MINISTERIO DEL PODER POPULAR PARA LA DEFENSA – FUERZA ARMADA NACIONAL BOLIVARIANA - GUARDIA NACIONAL BOLIVARIANA- DIRECCION DE OPERACIONES LABORATORIO CENTRAL - LABORATORIO REGIONAL N° 3 - DEPARTAMENTO DE BIOLOGÍA -MARACAIBO, DIECISIETE DE JUNIO DEL DOS MIL TRECE. 203° y 154°---

DICTAMEN PERICIAL BIOLÓGICO Y FISICO-QUÍMICO
NRO. CG-DO-LC-LR3-DB 13/0914
PAG. 1

I. DESIGNACIÓN: El Coronel, Jefe del Laboratorio Regional N° 3 de la Guardia Nacional Bolivariana; de conformidad con lo establecido en el artículo 224 del Código Orgánico Procesal Penal, designa a los ciudadanos: **TTE. MARLLING GUERRA MEDINA y la TTE. SUGHAES SÁNCHEZ TORRES** titulares de la cédula de identidad Nros **13.628.205** y **18.216.654**, respectivamente; para que en su condición de expertos y en virtud al requerimiento solicitado practiquen la experticia correspondiente.--

WAYME JOSÉ MARÍN OCANTO
C.I. V- 7.602.568

II. ACEPTACIÓN: Quienes suscriben, **TTE. MARLLING GUERRA MEDINA y la TTE. SUGHAES SÁNCHEZ TORRES,** expertos designados por esta Jefatura para colectar y realizar análisis microbiológicos y físico-químicos a la cantidad de dieciséis (16) muestras de agua; colectadas de cuatro (04) tanques plásticos de color celeste con capacidad de setecientos litros (700 L) c/u los cuales fueron llenados con agua del Lago de Maracaibo localizados en la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia. Dicho muestreo se lleva a cabo con la finalidad de realizar pruebas pilotos a un producto natural, a base de extractos de frutas, que funciona como sanitizante, agregado a los tanques de agua para observar el comportamiento del mismo. Las muestras se colectaron dando cumplimiento a la solicitud emitida por el Ciudadano **FÉLIX OSORIO GUZMÁN, MINISTRO DEL PODER POPULAR PARA LA ALIMENTACIÓN**, mediante oficio **Nro. 000516 DE FECHA 13MAY13**; rendimos a usted el siguiente Dictamen Pericial Biológico para los fines legales pertinentes, de conformidad con lo establecido en los Artículos 223 y 225 del Código Orgánico Procesal Penal.--

III. MOTIVO: La experticia ordenada tiene por objeto verificar si las muestras liquidas colectadas del sitio descrito, presentan algún tipo de contaminación desde el punto de vista microbiológico y físico-químico, según oficio **Nro. 000516 DE FECHA 13MAY13.**--------------

IV. EXPOSICION: Siendo los días 14MAY13, 17MAY13 y el 21MAY13, Expertos Adscritos al Laboratorio Regional Nro. 3, se trasladaron hasta las instalaciones de la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia.

DICTAMEN PERICIAL BIOLÓGICO Y FISICO-QUÍMICO
NRO. CG-DO-LC-LR3-DB 13/0914
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En presencia de la Ciudadana **VIANNET SEREIDA GUILLÉN C.I.V- 15.286.996** , Ingeniero Químico, Analista II Control de Calidad de mencionada procesadora, se procedió hacer la inspección del lugar y colectar las muestras en recipientes de vidrio previamente esterilizados, de color ámbar, cerrados con tapa de plástico de rosca, con capacidad de doscientos cincuenta mililitros (250ml) cada uno para realizar los análisis microbiológicos y envases plásticos de material sintético de color blanco cerrados con tapa de plástico, con capacidad de quinientos mililitros (500ml) cada uno para realizar los análisis fisico-quimicos. En este sentido, las muestras fueron colectadas de cuatro (04) tanques discriminados de la siguiente manera: dos (02) tanques identificados como PATRÓN, un (01) tanque identificado como 20 ml y un (01) tanque identificado como 40 ml, los envases se identificaron con los **Nros. 01 al 16** respectivamente y fueron trasladados en una cava de anime con hielo para la preservación de las mismas, debidamente precintada con cinta de embalar. Luego los expertos se trasladaron al Laboratorio Regional Nro. 3 para practicarle los análisis Fisico-Químicos y Microbiológicos a dichas muestras.---

A. DESCRIPCIÓN FÍSICA DE LAS MUESTRAS:

1. **Muestra 1:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 01**, y se anotaron los siguientes datos: "MUESTRA 1: COLECTADA DEL TANQUE (01) IDENTIFICADO COMO PATRÓN DE FECHA 14MAY13. HORA: 10:30 AM".---

2. **Muestra 2:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 02**, y se anotaron los siguientes datos: "MUESTRA 2: COLECTADA DEL TANQUE (02) IDENTIFICADO COMO PATRÓN DE FECHA 14MAY13. HORA: 10:35 AM".---

3. **Muestra 3:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 03** y se anotaron los siguientes datos: "MUESTRA 3: COLECTADA DEL TANQUE (01) IDENTIFICADO COMO PATRÓN DE FECHA 17MAY13. HORA: 16:00 PM".---

4. **Muestra 4:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 04** y se anotaron los siguientes datos: "MUESTRA 4: COLECTADA DEL TANQUE IDENTIFICADO COMO 20 ML DE FECHA 17MAY13. HORA: 16:10 PM".---

5. **Muestra 5:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 05** y se anotaron los siguientes datos: "MUESTRA 5: COLECTADA DEL TANQUE IDENTIFICADO COMO 40 ML DE FECHA 17MAY13. HORA: 16:20 PM".---

6. **Muestra 6:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 06** y se anotaron los siguientes datos:

DICTAMEN PERICIAL BIOLÓGICO Y FISICO-QUÍMICO
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15. **Muestra 15:** Líquido de apariencia turbia, con partículas en suspensión y partículas sedimentadas. El envase se identificó con el **Nro. 15** y se anotaron los siguientes datos: "*MUESTRA 15: COLECTADA DEL TANQUE IDENTIFICADO COMO 20 ML DE FECHA* 21MAY13. HORA: 10:20 AM".---

16. **Muestra 16:** Líquido de apariencia turbia, con partículas en suspensión y partículas sedimentadas. El envase se identificó con el **Nro. 16**, y se anotaron los siguientes datos: "MUESTRA 16: *COLECTADA DEL TANQUE IDENTIFICADO COMO 40 ML DE FECHA* 21MAY13. HORA: 10:40 AM".---

V. PERITACION: A fin de dar cumplimiento al pedimento formulado por este Departamento, los expertos designados hemos procedido a realizar los análisis microbiológicos y físico-químicos en la siguiente secuencia analítica: ---

A. ANÁLISIS FÍSICO-QUÍMICOS:

1. Color real: Determinado cuantitativamente por Espectrofotómetro HACH DR 2800.---------

2. pH: Determinado cuantitativamente utilizando un THERMO ORION MODEL 2300.-----------

3. Turbidez: Determinado cuantitativamente por Espectrofotómetro HACH DR 2800.-----------

4. Sólidos Flotantes: Determinados cualitativamente por observación directa de la muestra.------

5. Espuma: Determinada cualitativamente por observación directa de la muestra, luego de su adecuada homogeneización.---

B. ANÁLISIS MICROBIOLÓGICOS:

1. Ensayo de Orientación para Coliformes: Determinado por la Técnica de Tubos Múltiples de Fermentación con dilución, y son reportados en términos de Número Más Probable (NMP/100ml) de organismos presentes.---

2. Ensayo de Certeza para Coliformes totales: Determinado por la Técnica de Tubos Múltiples de Fermentación con dilución, y son reportados en términos de Número Más Probable (NMP/100ml) de organismos presentes.---

C. Resultados: De los ensayos y análisis practicados a las muestras colectadas se obtuvieron los siguientes resultados:

DICTAMEN PERICIAL BIOLÓGICO Y FISICO-QUÍMICO
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REPÚBLICA BOLIVARIANA DE VENEZUELA
Ministerio del Poder Popular para la Defensa
Guardia Nacional Bolivariana
MANDO DE OPERACIONES
Laboratorio Regional Nro. 3

"MUESTRA 6: COLECTADA DEL TANQUE (01) IDENTIFICADO COMO PATRÓN DE FECHA 21MAY13. HORA: 10:00 AM".--

7. **Muestra 7:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 07** y se anotaron los siguientes datos:

"MUESTRA 7: COLECTADA DEL TANQUE IDENTIFICADO COMO 20 ML DE FECHA 21MAY13. HORA: 10:10 AM".--

8. **Muestra 8:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 08**, y se anotaron los siguientes datos: "MUESTRA 8: *COLECTADA DEL TANQUE IDENTIFICADO COMO 40 ML DE FECHA* 21MAY13. HORA: 10:30 AM".--

9. **Muestra 9:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 09**, y se anotaron los siguientes datos: "*MUESTRA 9: COLECTADA DEL TANQUE (01) IDENTIFICADO COMO PATRÓN DE* FECHA 14MAY13. HORA: 10:40 AM".--

10. **Muestra 10:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 10**, y se anotaron los siguientes datos: "*MUESTRA 10: COLECTADA DEL TANQUE (02) IDENTIFICADO COMO PATRÓN DE* FECHA 14MAY13. HORA: 10:45 AM".--

11. **Muestra 11:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 11** y se anotaron los siguientes datos: "*MUESTRA 11: COLECTADA DEL TANQUE (01) IDENTIFICADO COMO PATRÓN DE* FECHA 17MAY13. HORA: 16:10 PM".--

12. **Muestra 12:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 12** y se anotaron los siguientes datos: "*MUESTRA 12: COLECTADA DEL TANQUE IDENTIFICADO COMO 20 ML DE FECHA* 17MAY13. HORA: 16:20 PM".--

13. **Muestra 13:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 13** y se anotaron los siguientes datos: "*MUESTRA 13: COLECTADA DEL TANQUE IDENTIFICADO COMO 40 ML DE FECHA* 17MAY13. HORA: 16:30 PM".--

14. **Muestra 14:** Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 14** y se anotaron los siguientes datos: "*MUESTRA 14: COLECTADA DEL TANQUE (01) IDENTIFICADO COMO PATRÓN DE* FECHA 21MAY13. HORA: 10:10 AM".--

DICTAMEN PERICIAL BIOLÓGICO Y FISICO-QUÍMICO
NRO. CG-DO-LC-LR3-DB 13/0914
PAG. 5

REPUBLICA BOLIVARIANA DE VENEZUELA
Ministerio del Poder Popular para la Defensa
Guardia Nacional Bolivariana
COMANDO DE OPERACIONES
Laboratorio Regional Nro. 3

Tabla de resultados de los Análisis Fisicoquímicos:

Muestra	pH 25 °C	Turbiedad	[a] Color Real (U-Pt/Co)	Sólidos Flotantes	*Espuma*
01	8,54	31,5	179,3	Presentes	Ausente
02	9,11	27,1	143,3	Presentes	Ausente
03	8,94	31,0	193,0	Presentes	Ausente
04	8,73	22,4	142,0	Presentes	Ausente
05	8,19	28,6	173,3	Presentes	Ausente
06	8,03	13,0	76,3	Presentes	Ausente
07	7,58	14,7	90,6	Presentes	Ausente
08	7,22	23,4	140,3	Presentes	Ausente
Límites Máximos o Rangos	**6 - 9**	*	**500 Unidades de Pt/Co**	**Ausentes**	**Ausentes**

* No existen valores establecidos para la Turbiedad de descargas a cuerpos de agua.-------------------

a: Unidad de Platino/Cobalto--

Tabla de resultados de los Análisis Microbiológicos

Muestras	*Coliformes totales/en 100ml NMP*
09	> 24.000
10	> 24.000
11	> 24.000
12	> 24.000
13	> 24.000
14	> 24.000
15	> 24.000
16	> 24.000
***Límite Máximo**	< 5.000

(*) De acuerdo a lo establecido en el Decreto Presidencial número 883 publicado en la Gaceta Oficial de la República de Venezuela N° 5.021 del 18DIC95. **Artículo 10** (De las descargas a cuerpos de agua). Números más probable (NMP) de organismos Coliformes totales no mayor de 1000 por cada 100 ml, en el 90% de una serie de muestras consecutivas y en ningún caso será superior a 5000 por cada 100 ml. ---

DICTAMEN PERICIAL BIOLÓGICO Y FISICO-QUÍMICO
NRO. CG-DO-LC-LR3-DB 13/0914
PAG. 6

VI. CONCLUSIONES: En base al estudio y evaluación de los resultados obtenidos en los análisis fisico-químicos y microbiológicos realizados a dieciséis (16) muestras liquidas identificadas con los Nros. 01 al 16, colectadas de cuatro (04) tanques llenados con agua del Lago de Maracaibo localizados en la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia, dando cumplimiento a las instrucciones del Ciudadano **FÉLIX OSORIO GUZMÁN, MINISTRO DEL PODER POPULAR PARA LA ALIMENTACIÓN**, mediante oficio Nro. 000516 DE FECHA 13MAY13, se concluye que:

A. Las muestras de aguas provenientes del Lago de Maracaibo, identificadas con los **Nros. 01 al 08**, colectadas de los cuatro (04) tanques (ver ítem IV-A, descripción física de las muestras) de la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia, se obtuvo que los parámetros fisicoquímicos de pH, Color Real y Espuma **CUMPLEN con los limites máximos permitidos en el Artículo 10, Sección III, Capítulo III (De las descargas a cuerpos de agua), de las Normas para la Clasificación y el Control de la Calidad de los Cuerpos de Agua y Vertidos o Efluentes liquidos**, del Decreto Presidencial número 883, publicado en la Gaceta Oficial de la República de Venezuela Nro. 5.021 del 18DIC95.--

B. Las muestras de aguas provenientes del Lago de Maracaibo, identificadas con los **Nros. 01 al 08**, colectadas de los cuatro (04) tanques (ver ítem IV-A, descripción física de las muestras) de la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia, se obtuvo que el parámetro fisicoquímico de Sólidos Flotantes NO **CUMPLE con los límite máximo permitido en el Artículo 10, Sección III, Capítulo III (De las descargas a cuerpos de agua), de las Normas para la Clasificación y el Control de la Calidad de los Cuerpos de Agua y Vertidos o Efluentes liquidos**, del Decreto Presidencial número 883, publicado en la Gaceta Oficial de la República de Venezuela Nro. 5.021 del 18DIC95.--

C. Por otra parte, las muestras de aguas provenientes del Lago de Maracaibo, identificadas con los **Nros. 01 al 08**, no existen valores establecidos para la Turbiedad de descargas a cuerpos de agua.--

D. Desde el punto de vista microbiológico, las muestras de agua, identificadas con los números **"09 al 16"**, colectadas de los cuatro (04) tanques (ver ítem IV-A, descripción física de las muestras) de la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia, ***no cumplen*** con los limites máximos permitidos y exigidos en el Artículo 10 (De las descargas a cuerpos de agua), de acuerdo a la clasificación establecida por las Normas Técnicas de Clasificación y Control de la Calidad de los Cuerpos de Agua y Vertidos o Efluentes Liquidos, del Decreto Presidencial número 883, publicado en la Gaceta Oficial de la República de Venezuela Nro. 5.021 del 18DIC95.--------------------

DICTAMEN PERICIAL BIOLÓGICO Y FISICO-QUÍMICO
NRO. CG-DO-LC-LR3-DB 13/0914
PAG. 7

E. Con lo antes expuesto, damos por concluidas nuestras actuaciones periciales y cumplimos con consignar el presente Dictamen Pericial que consta de siete (07) folios útiles e informar que las muestras se consumieron en la realización de los análisis.---------------------------------

Los expertos,

REPUBLICA BOLIVARIANA DE VENEZUELA
Ministerio del Poder Popular para la Defensa
Guardia Nacional Bolivariana
COMANDO DE OPERACIONES

MARLLING GUERRA MEDINA	**SUGHAES SÁNCHEZ TORRES**
C.I: 13.628.205	**C.I:18.216.654**
TTE. LCDA EN BIOLOGÍA	**TTE. LCDA EN QUÍMICA**

INZIT Laboratory Results

Gobierno Bolivariano de Venezuela | Ministerio del Poder Popular para Ciencia, Tecnología e Innovación | Fundación Instituto Zuliano de Investigaciones Tecnológicas (INZIT)

Rif. G-20005459-0

Fundación Instituto de Investigaciones Tecnológicas

INZIT

INFORME DE RESULTADOS

ANÁLISIS DE AGUA

ATENCION: ALEJANDRO ADAMES GONZALEZ

ORDEN DE TRABAJO Nº 1688
SERVICIOS ESPECIALES

La Cañada de Urdaneta, 05 de junio del 2013

Realizado por:	Revisado por:
TSU Guillermo Valbuena	Lcda. Lisis Ospino
Laboratorio de Ambiente	Coordinador de Servicios Técnicos

Dirección: Km 15, carretera vía a la Cañada de Urdaneta, entrando por el patio de tanques Punta de Palmas, Sector Palmarejo Viejo.
Teléfonos: (0261) 791.5371 / 7913769 FAX: (0261) 791.0952. Sitio web: www.inzit.gob.ve, email: lospino@inzit.gob.ve

Rif. G-20005459-0

La Cañada de Urdaneta, 05 de junio del 2013

USUARIO: ALEJANDRO ADAMES GONZALEZ
ATENCIÓN: ALEJANDRO ADAMES GONZALEZ

1. INTRODUCCIÓN

El señor Alejandro Adames Gonzalez ha solicitado realizar la caracterización de aguas procedente del lago de Maracaibo, al Instituto Zuliano de Investigaciones Tecnológicas (INZIT)

2. IDENTIFICACIÓN DE MUESTRAS:

Se captaron por el personal del INZIT,cuatro (04) muestras de agua el día 17 de mayo del presente año, y se codificaron bajo la orden Nº 1688 e identificada como se índica en la Tabla 1.

Tabla 1. Identificación de Muestras

Código INZIT	Descripción
1688-02-13-12495	MUESTRA PATRON
1688-02-13-12496	MUESTRA 20 MTS
1688-02-13-12497	MUESTRA 40 MTS
1688-02-13-12498	MUESTRA DEL LAGO

3. METODOLOGÍA:

La muestra de agua se analizó siguiendo los procedimientos descritos en el "Standard Methods for the Examination of Water and Wastewater" 20th Edition.

4. PARÁMETROS ANALIZADOS:

Los requeridos por el usuario, Demanda bioqumica de oxigeno, Demanda quimica de oxigeno, Fosfatos, Nitritos, Nitratos, Oxigeno disuelto, Solidos sedimentables, Solidos totales, Sulfatos y Ph. se realiza según el Art.10 decreto 883 referente a la descarga a cuerpos de agua cuyo criterio establece unos valores máximos de calidad de vertidos líquidos a ser descargados en forma directa o indirecta a ríos, estuarios, lagos y embalses.

5. MÉTODO DE MUESTREO:

Las muestras se captaron en envases plásticos y de vidrio de diferentes capacidades (500 mL y 1L). Las muestras se preservaron con los reactivos necesarios (ácido etilendiaminotetraacético

Realizado por:
TSU Guillermo Valbuena
Laboratorio de Ambiente

Revisado por:
Lcdo. Luis Espino
Coordinador de Servicios Técnicos

Dirección: Km 15, carretera vía a la Cañada de Urdaneta, entrando por el patio de tanques Punta de Palmas, Sector Palmarejo Viejo.
Teléfonos: (0261) 791.5371 / 7913769 FAX: (0261) 791.0952. Sitio web: www.inzit.gob.ve, email: lospino@inzit.gob.ve

Rif. G-20005459-0

EDTA, ácido sulfúrico H_2SO_4, ácido nítrico HNO_3, hidróxido de sodio *NaOH*, acetato de zinc $(CH_3COO)_2Zn$) para evitar fenómenos de adsorción de elementos traza en la superficie de los envases en algunos casos, y en otros, adecuar las condiciones físico-químicas y evitar pérdida o contaminación del analito durante el almacenaje y transporte. Una vez tomadas y preservadas con los reactivos correspondientes, el conjunto de muestras fue conservado a temperatura controlada (~4 °C) y trasladadas en el menor tiempo posible al laboratorio para los análisis respectivos.

6. RESULTADOS:

Los resultados obtenidos en las muestras evaluadas, son indicados en la tabla de resultados anexa.

7. REFERENCIA BIBLIOGRÁFICA:

1. "Standard Methods for the Examination of Water and Wastewater" 20th edition.
2. República de Venezuela. Decreto 883 "**Normas para la clasificación y el control de la calidad de e agua.**. Gaceta oficial Nº 4.899.19/05/1995.

Realizado por:
TSU Guillermo Valbuena
Laboratorio de Ambiente

Revisado por:
Lcdo. Luis Ospino
Coordinador de Servicios Técnicos

Dirección: Km 15, carretera vía a la Cañada de Urdaneta, entrando por el patio de tanques Punta de Palmas, Sector Palmarejo Viejo.
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RIF. G-20005459-0

CERTIFICADOS DE ENSAYO

Realizado por:
TSU Guillermo Valbuena
Laboratorio de Ambiente

Revisado por:
Lcdo. Luis Ospino
Coordinador de Servicios Técnicos

Dirección: Km 15, carretera vía a la Cañada de Urdaneta, entrando por el patio de tanques Punta de Palmas, Sector Palmarejo Viejo.
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COORDINACION DE SERVICIOS TECNICOS
LABORATORIO DE AMBIENTE
CERTIFICADO DE ENSAYO

LABORATORIO RESPONSABLE	SERVICIOS ESPECIALES
SUPERVISOR RESPONSABLE	MAGLE MARIA VILLASMIL MARTINEZ
USUARIO	ALEJANDRO ADAMES GONZALEZ
No. ORDEN USUARIO	38833338 No. ODT 1688
FECHA EMISION ORDEN	07/06/2013
CONTACTO (USUARIO)	VIANNET FEREIRA
CORREO ELECTRONICO (USUARIO)	vfereira@gmailcom

CODIGO MUESTRA INZIT	1688-02-13-12495		
CODIGO MUESTRA USUARIO	MUESTRA PATRON		
TIPO DE MUESTRA	AGUAS		
FECHA DE CAPTACIÓN	17/05/2013		
SITIO DE CAPTACIÓN	PLANTA PROCESADORA		
HORA INICIO	1:10 pm	HORA FINAL	
COORDENADAS	N:	W:	

Analisis	Descripción	ARTÍCULO 10 DECRETO 883	Resultado	Comparación	Metodo
LA-0010L	DETERMINACIÓN DE DEMANDA BIOQUIMICA DE OXIGENO	<= 60 mg/L	9.8_mg/L	CUMPLE	SM5210B
LA-0011L	DETERMINACIÓN DE DEMANDA QUIMICA DE OXIGENO	<= 350 mg/L	236_mg/L	CUMPLE	SM5220B
LA-0035L	DETERMINACIÓN DE FOSFATOS	---	1.38 mg/L	---	SMWW4500P
LA-0039L	DETERMINACIÓN DE NITRATOS	---	1.58 mg/L	---	SMWW419D
LA-0040L	DETERMINACIÓN DE NITRITOS	---	0.78 mg/L	---	SM4500NO2
LA-0046L	DETERMINACIÓN DE OXIGENO DISUELTO	---	3.5 mg/L	---	SM4500O
LA-0052L	DETERMINACIÓN DE SOLIDOS SEDIMENTABLES	<= 1 ml/L	<0.5_ml/L	CUMPLE	SM240F
LA-0054L	DETERMINACIÓN DE SOLIDOS TOTALES	---	3144 mg/L	---	SM2540
LA-0056L	DETERMINACIÓN DE SULFATOS	<= 1000 mg/L	326_mg/L	CUMPLE	SM4500SO4
LA-0066L	MEDICION DE PH	6 - 9	7.36	CUMPLE	SM4500HB

NOTA: Los resultados donde no aparecen comparacion se dan como informativos.

LABORATORIO DE AMBIENTE
Supervisor: --- CARLOS ARTURO GUERRERO

COORDINADOR DE SERVICIOS TECNICOS
Lcdo. LUIS OSPINO

COORDINACION DE SERVICIOS TECNICOS
LABORATORIO DE AMBIENTE
CERTIFICADO DE ENSAYO

CODIGO MUESTRA INZIT	1688-02-13-12496
CODIGO MUESTRA USUARIO	MUESTRA 20 MLS
TIPO DE MUESTRA	AGUAS
FECHA DE CAPTACIÓN	17/05/2013
SITIO DE CAPTACIÓN	PLANTA PROCESADORA
HORA INICIO	2:10 pm HORA FINAL
COORDENADAS	N: W:

Analisis	Descripción	ARTÍCULO 10 DECRETO 883	Resultado	Comparación	Metodo
LA-0010L	DETERMINACIÓN DE DEMANDA BIOQUIMICA DE OXIGENO	<= 60 mg/L	4.7_mg/L	CUMPLE	SM5210B
LA-0011L	DETERMINACIÓN DE DEMANDA QUIMICA DE OXIGENO	<= 350 mg/L	223_mg/L	CUMPLE	SM5220B
LA-0035L	DETERMINACIÓN DE FOSFATOS	- - -	0.45 mg/L	- - -	SMWW4500P
LA-0039L	DETERMINACIÓN DE NITRATOS	- - -	1.18 mg/L	- - -	SMWW419D
LA-0040L	DETERMINACIÓN DE NITRITOS	- - -	0.14 mg/L	- - -	SM4500NO2
LA-0046L	DETERMINACIÓN DE OXIGENO DISUELTO	- - -	3.7 mg/L	- - -	SM4500O
LA-0052L	DETERMINACIÓN DE SOLIDOS SEDIMENTABLES	<= 1 ml/L	<0.5_ml/L	CUMPLE	SM240F
LA-0054L	DETERMINACIÓN DE SOLIDOS TOTALES	- - -	3132 mg/L	- - -	SM2540
LA-0056L	DETERMINACIÓN DE SULFATOS	<= 1000 mg/L	309_mg/L	CUMPLE	SM4500SO4
LA-0066L	MEDICION DE PH	6 - 9 _	7.26	CUMPLE	SM4500HB

NOTA: Los resultados donde no aparecen comparacion se dan como informativos.

LABORATORIO DE AMBIENTE
Supervisor: CARLOS ARTURO GUERRERO

COORDINADOR DE SERVICIOS TECNICOS
Lcdo. LUIS OSPINO

Gobierno Bolivariano de Venezuela | Ministerio del Poder Popular para Ciencia, Tecnología e Innovación | Fundación Instituto Zuliano de Investigaciones Tecnológicas (INZIT)

Proceso: Certificado de Ensayo

Codigo Control ST-RT-010-03
Revision No. 0

PAGINA 3 DE 5
Fecha Aprobacion Junio 2010

COORDINACION DE SERVICIOS TECNICOS
LABORATORIO DE AMBIENTE
CERTIFICADO DE ENSAYO

CODIGO MUESTRA INZIT	1688-02-13-12497		
CODIGO MUESTRA USUARIO	MUESTRA 40 MLS		
TIPO DE MUESTRA	AGUAS		
FECHA DE CAPTACIÓN	17/05/2013		
SITIO DE CAPTACIÓN	PLANTA PROCESADORA		
HORA INICIO	2:10 pm	HORA FINAL	
COORDENADAS	N:	W:	

Analisis	Descripción	ARTÍCULO 10 DECRETO 883	Resultado	Comparación	Metodo
LA-0010L	DETERMINACIÓN DE DEMANDA BIOQUIMICA DE OXIGENO	<= 60 mg/L	5.5_mg/L	CUMPLE	SM5210B
LA-0011L	DETERMINACIÓN DE DEMANDA QUIMICA DE OXIGENO	<= 350 mg/l	223_mg/L	CUMPLE	SM5220B
LA-0035L	DETERMINACIÓN DE FOSFATOS	- - -	1.10 mg/L	- - -	SMWW4500P
LA-0039L	DETERMINACIÓN DE NITRATOS	- - -	1.37 mg/L	- - -	SMWW419D
LA-0040L	DETERMINACIÓN DE NITRITOS	- - -	0.20 mg/L	- - -	SM4500NO2
LA-0046L	DETERMINACIÓN DE OXIGENO DISUELTO	- - -	3.9 mg/L	- - -	SM4500O
LA-0052L	DETERMINACIÓN DE SOLIDOS SEDIMENTABLES	<= 1 ml/L	<0.5_ml/L	CUMPLE	SM240F
LA-0054L	DETERMINACIÓN DE SOLIDOS TOTALES	- - -	2978 mg/L	- - -	SM2540
LA-0056L	DETERMINACIÓN DE SULFATOS	<= 1000 mg/L	295_mg/L	CUMPLE	SM4500SO4
LA-0066L	MEDICION DE PH	6 - 9_	7.20	CUMPLE	SM4500HB

NOTA: Los resultados donde no aparecen comparacion se dan como informativos.

LABORATORIO DE AMBIENTE
Supervisor: CARLOS ARTURO GUERRERO

COORDINADOR DE SERVICIOS TECNICOS
Lcdo. LUIS OSPINO

Constructores Socialismo

COORDINACION DE SERVICIOS TECNICOS
LABORATORIO DE AMBIENTE
CERTIFICADO DE ENSAYO

CODIGO MUESTRA INZIT	1688-02-13-12498		
CODIGO MUESTRA USUARIO	AGUA DEL LAGO		
TIPO DE MUESTRA	AGUAS		
FECHA DE CAPTACIÓN	17/05/2013		
SITIO DE CAPTACIÓN	PLANTA PROCESADORA		
HORA INICIO	2:10 pm	HORA FINAL	
COORDENADAS	N:	W:	

Analisis	Descripción	ARTÍCULO 10 DECRETO 883	Resultado	Comparación	Metodo
LA-0010L	DETERMINACIÓN DE DEMANDA BIOQUIMICA DE OXIGENO	<= 60 mg/L	11.7_mg/L	CUMPLE	SM5210B
LA-0011L	DETERMINACIÓN DE DEMANDA QUIMICA DE OXIGENO	<= 350 mg/L	72_mg/L	CUMPLE	SM5220B
LA-0035L	DETERMINACIÓN DE FOSFATOS	- - -	0.36 mg/L	- - -	SMWW4500P
LA-0039L	DETERMINACIÓN DE NITRATOS	- - -	1.26 mg/L	- - -	SMWW419D
LA-0040L	DETERMINACIÓN DE NITRITOS	- - -	0.14 mg/L	- - -	SM4500NO2
LA-0046L	DETERMINACIÓN DE OXIGENO DISUELTO	- - -	5.1 mg/L	- - -	SM4500O
LA-0052L	DETERMINACIÓN DE SOLIDOS SEDIMENTABLES	<= 1 ml/L	<0.5_ml/L	CUMPLE	SM240F
LA-0054L	DETERMINACIÓN DE SOLIDOS TOTALES	- - -	2802 mg/L	- - -	SM2540
LA-0056L	DETERMINACIÓN DE SULFATOS	<= 1000 mg/L	258_mg/L	CUMPLE	SM4500SO4
LA-0066L	MEDICION DE PH	6 - 9	7.42	CUMPLE	SM4500HB

NOTA: Los resultados donde no aparecen comparacion se dan como informativos.

LABORATORIO DE AMBIENTE
Supervisor: CARLOS ARTURO GUERRERO

COORDINADOR DE SERVICIOS TECNICOS
Lcdo. LUIS OSPINO

COORDINACION DE SERVICIOS TECNICOS
LABORATORIO DE AMBIENTE
CERTIFICADO DE ENSAYO

Rif. G-20009459-0

INFORME DE RESULTADOS

ANÁLISIS DE AGUA

ATENCION: ALEJANDRO ADAMES GONZALEZ

ORDEN DE TRABAJO Nº 1692
SERVICIOS ESPECIALES

La Cañada de Urdaneta, 05 de junio del 2013

Realizado por:
TSU Guillermo Valbuena
Laboratorio de Ambiente

Revisado por:
Lcdo. Luis Ospino
Coordinador de Servicios Técnicos

Dirección: Km 15, carretera vía a la Cañada de Urdaneta, entrando por el patio de tanques Punta de Palmas, Sector Palmarejo Viejo.
Teléfonos: (0261) 791.5371 / 7913769 FAX: (0261) 791.0952. Sitio web: www.inzit.gob.ve, email: lospino@inzit.gob.ve

La Cañada de Urdaneta, 05 de junio del 2013

USUARIO: ALEJANDRO ADAMES GONZALEZ
ATENCIÓN: ALEJANDRO ADAMES GONZALEZ

1. INTRODUCCIÓN

El señor Alejandro Adames Gonzalez ha solicitado realizar la caracterización de aguas procedente del lago de Maracaibo, al Instituto Zuliano de Investigaciones Tecnológicas (INZIT)

2. IDENTIFICACIÓN DE MUESTRAS:

Se capto por el personal del INZIT, tres (03) muestras de agua el día 23 de mayo del presente año, y se codifico bajo la orden Nº 1692 e identificada como se indica en la Tabla 1.

Tabla 1. Identificación de Muestras

Código INZIT	Descripción
1692-02-13-12519	MUESTRA PATRON
1692-02-13-12520	MUESTRA 20 MTS
1692-02-13-12521	MUESTRA 40 MTS

3. METODOLOGÍA:

La muestra de agua se analizó siguiendo los procedimientos descritos en el "Standard Methods for the Examination of Water and Wastewater" 20th Edition.

4. PARÁMETROS ANALIZADOS:

Los requeridos por el usuario, Demanda bioqumica de oxigeno, Demanda quimica de oxigeno, Fosfatos, Nitritos, Nitratos, Oxigeno disuelto, Solidos sedimentables, Solidos totales, Sulfatos y Ph. se realiza según el Art.10 decreto 883 referente a la descarga a cuerpos de agua cuyo criterio establece unos valores máximos de calidad de vertidos líquidos a ser descargados en forma directa o indirecta a ríos, estuarios, lagos y embalses.

5. MÉTODO DE MUESTREO:

Las muestras se captaron en envases plásticos y de vidrio de diferentes capacidades (500 mL y 1L). Las muestras se preservaron con los reactivos necesarios (ácido etilendiaminotetraacético *EDTA*, ácido sulfúrico H_2SO_4, ácido nítrico HNO_3, hidróxido de sodio *NaOH*, acetato de zinc

Realizado por:
TSU Guillermo Valbuena
Laboratorio de Ambiente

Revisado por:
Lcdo. Luis Ospino
Coordinador de Servicios Técnicos

Dirección: Km 15, carretera vía a la Cañada de Urdaneta, entrando por el patio de tanques Punta de Palmas, Sector Palmarejo Viejo.
Teléfonos: (0261) 791.5371 / 7913769 FAX: (0261) 791.0952. Sitio web: www.inzit.gob.ve, email: lospino@inzit.gob.ve

($(CH_3COO)_2Zn$) para evitar fenómenos de adsorción de elementos traza en la superficie de los envases en algunos casos, y en otros, adecuar las condiciones físico-químicas y evitar pérdida o contaminación del analito durante el almacenaje y transporte. Una vez tomadas y preservadas con los reactivos correspondientes, el conjunto de muestras fue conservado a temperatura controlada (~4 °C) y trasladadas en el menor tiempo posible al laboratorio para los análisis respectivos.

6. RESULTADOS:

Los resultados obtenidos en las muestras evaluadas, son indicados en la tabla de resultados anexa.

7. REFERENCIA BIBLIOGRÁFICA:

1. "Standard Methods for the Examination of Water and Wastewater" 20th edition.
2. República de Venezuela. Decreto 883 **"Normas para la clasificación y el control de la calidad de e agua.**. Gaceta oficial Nº 4.899.19/05/1995.

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Rif. G-20005459-0

CERTIFICADOS DE ENSAYO

Realizado por:
TSU Guillermo Valbuena
Laboratorio de Ambiente

Revisado por:
Lcdo. Luis Ospino
Coordinador de Servicios Técnicos

Dirección: Km 15, carretera vía a la Cañada de Urdaneta, entrando por el patio de tanques Punta de Palmas, Sector Palmarejo Viejo.
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Gobierno Bolivariano de Venezuela | Ministerio del Poder Popular para Ciencia, Tecnología e Innovación | Fundación Instituto Zuliano de Investigaciones Tecnológicas (INZIT)

Proceso: Certificado de Ensayo | Codigo Control ST-RT-010-03 Revision No. 0 | PAGINA 2 DE 4 Fecha Aprobacion Junio 2010

COORDINACION DE SERVICIOS TECNICOS
LABORATORIO DE AMBIENTE
CERTIFICADO DE ENSAYO

CODIGO MUESTRA INZIT	1692-02-13-12520		
CODIGO MUESTRA USUARIO	MUESTRA A 20 MTS		
TIPO DE MUESTRA	AGUAS		
FECHA DE CAPTACIÓN	21/05/2013		
SITIO DE CAPTACIÓN	PLANTA PROCESADORA		
HORA INICIO	1:00 pm	HORA FINAL	3:30 pm
COORDENADAS	N: 10º32'33.5"	W: 071º31'27.1"	

Analisis	Descripción	ARTÍCULO 10 DECRETO 883	Resultado	Comparación	Metodo
LA-0010L	DETERMINACIÓN DE DEMANDA BIOQUIMICA DE OXIGENO	<= 60 mg/L	<2_mg/L	CUMPLE	SM5210B
LA-0011L	DETERMINACIÓN DE DEMANDA QUIMICA DE OXIGENO	<= 350 mg/L	190_mg/L	CUMPLE	SM5220B
LA-0035L	DETERMINACIÓN DE FOSFATOS	- - -	0.45 mg/L	- - -	SMWW4500P
LA-0039L	DETERMINACIÓN DE NITRATOS	- - -	1.54 mg/L	- - -	SMWW419D
LA-0040L	DETERMINACIÓN DE NITRITOS	- - -	0.54 mg/L	- - -	SM4500NO2
LA-0046L	DETERMINACIÓN DE OXIGENO DISUELTO	- - -	3.7 mg/L	- - -	SM4500O
LA-0052L	DETERMINACIÓN DE SOLIDOS SEDIMENTABLES	<= 1 ml/L	<0.5_ml/L	CUMPLE	SM240F
LA-0054L	DETERMINACIÓN DE SOLIDOS TOTALES	- - -	3004 mg/L	- - -	SM2540
LA-0056L	DETERMINACIÓN DE SULFATOS	<= 1000 mg/L	244_mg/L	CUMPLE	SM4500SO4
LA-0066L	MEDICION DE PH	6 - 9_	7.04	CUMPLE	SM4500HB

NOTA: Los resultados donde no aparecen comparacion se dan como informativos.

LABORATORIO DE AMBIENTE
Supervisor: CARLOS ARTURO GUERRERO

COORDINADOR DE SERVICIOS TECNICOS
Lcdo. LUIS OSPINO

Constructores Socialismo

COORDINACION DE SERVICIOS TECNICOS
LABORATORIO DE AMBIENTE
CERTIFICADO DE ENSAYO

LABORATORIO RESPONSABLE	LABORATORIO DE AMBIENTE
SUPERVISOR RESPONSABLE	CARLOS ARTURO GUERRERO
USUARIO	ALEJANDRO ADAMES GONZALEZ
No. ORDEN USUARIO	38833338 — No. ODT 1692
FECHA EMISION ORDEN	07/06/2013
CONTACTO (USUARIO)	VIANNET FEREIRA
CORREO ELECTRONICO (USUARIO)	vfereira@gmailcom

CODIGO MUESTRA INZIT	1692-02-13-12519		
CODIGO MUESTRA USUARIO	MUESTRA PATRON		
TIPO DE MUESTRA	AGUAS		
FECHA DE CAPTACIÓN	21/05/2013		
SITIO DE CAPTACIÓN	PLANTA PROCESADORA		
HORA INICIO	1:00 pm	HORA FINAL	3:30 pm
COORDENADAS	N: 10º32'33.5"	W: 071º31'27.1"	

Analisis	Descripción	ARTÍCULO 10 DECRETO 883	Resultado	Comparación	Metodo
LA-0010L	DETERMINACIÓN DE DEMANDA BIOQUIMICA DE OXIGENO	<= 60 mg/L	2_mg/L	CUMPLE	SM5210B
LA-0011L	DETERMINACIÓN DE DEMANDA QUIMICA DE OXIGENO	<= 350 mg/L	216_mg/L	CUMPLE	SM5220B
LA-0035L	DETERMINACIÓN DE FOSFATOS	- - -	1.16 mg/L	- - -	SMWW4500P
LA-0039L	DETERMINACIÓN DE NITRATOS	- - -	3.24 mg/L	- - -	SMWW419D
LA-0040L	DETERMINACIÓN DE NITRITOS	- - -	2.44 mg/L	- - -	SM4500NO2
LA-0046L	DETERMINACIÓN DE OXIGENO DISUELTO	- - -	3.6 mg/L	- - -	SM4500O
LA-0052L	DETERMINACIÓN DE SOLIDOS SEDIMENTABLES	<= 1 ml/L	<0.5_ml/L	CUMPLE	SM240F
LA-0054L	DETERMINACIÓN DE SOLIDOS TOTALES	- - -	3128 mg/L	- - -	SM2540
LA-0056L	DETERMINACIÓN DE SULFATOS	<= 1000 mg/L	298_mg/L	CUMPLE	SM4500SO4
LA-0066L	MEDICION DE PH	6 - 9	7.02	CUMPLE	SM4500HB

NOTA: Los resultados donde no aparecen comparacion se dan como informativos.

LABORATORIO DE AMBIENTE
Supervisor: --- CARLOS ARTURO GUERRERO

COORDINADOR DE SERVICIOS TECNICOS
Lcdo. LUIS OSPINO

COORDINACION DE SERVICIOS TECNICOS
LABORATORIO DE AMBIENTE
CERTIFICADO DE ENSAYO

CODIGO MUESTRA INZIT	1692-02-13-12521		
CODIGO MUESTRA USUARIO	MUESTRA A 40 MTS		
TIPO DE MUESTRA	AGUAS		
FECHA DE CAPTACIÓN	21/05/2013		
SITIO DE CAPTACIÓN	PLANTA PROCESADORA		
HORA INICIO	1:00 pm	HORA FINAL	3:30 pm
COORDENADAS	N: 10º32'33.5"	W: 071º31'27.1"	

Analisis	Descripción	ARTÍCULO 10 DECRETO 883	Resultado	Comparación	Metodo
LA-0010L	DETERMINACIÓN DE DEMANDA BIOQUIMICA DE OXIGENO	<= 60 mg/L	2_mg/L	CUMPLE	SM5210B
LA-0011L	DETERMINACIÓN DE DEMANDA QUIMICA DE OXIGENO	<= 350 mg/L	197_mg/L	CUMPLE	SM5220B
LA-0035L	DETERMINACIÓN DE FOSFATOS	- - -	1.02 mg/L	- - -	SMWW4500P
LA-0039L	DETERMINACIÓN DE NITRATOS	- - -	1.73 mg/L	- - -	SMWW419D
LA-0040L	DETERMINACIÓN DE NITRITOS	- - -	1.02 mg/L	- - -	SM4500NO2
LA-0046L	DETERMINACIÓN DE OXIGENO DISUELTO	- - -	3.7 mg/L	- - -	SM4500O
LA-0052L	DETERMINACIÓN DE SOLIDOS SEDIMENTABLES	<= 1 ml/L	<0.5_ml/L	CUMPLE	SM240F
LA-0054L	DETERMINACIÓN DE SOLIDOS TOTALES	- - -	3062 mg/L	- - -	SM2540
LA-0056L	DETERMINACIÓN DE SULFATOS	<= 1000 mg/L	289_mg/L	CUMPLE	SM4500SO4
LA-0066L	MEDICION DE PH	6 - 9	7.08	CUMPLE	SM4500HB

NOTA: Los resultados donde no aparecen comparacion se dan como informativos.

LABORATORIO DE AMBIENTE
Supervisor: --- CARLOS ARTURO GUERRERO

COORDINADOR DE SERVICIOS TECNICOS
Lcdo. LUIS OSPINO

COORDINACION DE SERVICIOS TECNICOS
LABORATORIO DE AMBIENTE
CERTIFICADO DE ENSAYO

PDVSA

ALMACENAMIENTO TRATAMIENTO Y TRANSPORTE DE CRUDO
TRATAMIENTO Y LABORATORIO
LABORATORIO DE PETROLEO
TIA JUANA

INFORME DE ENSAYO

Solicitado por: MANUEL ANTONIO PEREZ TRUJILLO
Fecha de Toma: 06/06/2013
Fecha de Análisis 06/06/2013

RESULTADO DE ANALISIS FISICOQUÍMICOS AGUA DEL LAGO 06/06/2013

MUESTRA	CONTROL 06/06/13	DOSIS 20ml 06/06/13	DOSIS 40ml 06/06/13
FECHA DE TOMA	06/06/2013	06/06/2013	06/06/2013
FECHA DE ANALISIS	06/06/2013	06/06/2013	06/06/2013
Temp (°F)	78	78	78
pH	8,37	7,85	7,84
ALC.	52,60	51,62	45,13
Na+	778,47	794,18	635,18
Resistividad	3,35	3,38	4,01
Conductividad	NO DETECTADO	NO DETECTADO	NO DETECTADO
CO3=	5,3	NO DETECTADO	NO DETECTADO
HCO3-	58,80	57,60	55,08
Cl-	1415	1398,00	1188,49
Ca++	26,21	25,13	20,40
Mg++	77,00	62,78	75,80
DT	389,60	354,25	354,25
SiO2	13,20	7,94	12,90
SO4=	26,00	24,00	22,00
Fe	N / D	N / D	N / D
Sol. Susp	6,00	6,00	4,00
indice de STIFF 140°F	0,11	-0,44	-0,54
indice de STIFF 180°F	0,45	-0,10	-0,21
indice de STIFF 200°F	0,64	0,09	-0,02
Tendencia de STIFF 140 °F	INCRUSTANTE	NO INCRUSTANTE	NO INCRUSTANTE
Tendencia de STIFF 180 °F	INCRUSTANTE	NO INCRUSTANTE	NO INCRUSTANTE
Tendencia de STIFF 200 °F	INCRUSTANTE	INCRUSTANTE	NO INCRUSTANTE

Realizado por
NESTOR GRANDA

Validado por
NOLBERTO PIÑA LIDER DE LABORATORIO

Revisado por
GERARDO OSORIO

Aprobado por
GERARDO HERNANDEZ GTE. DE LAB. C.O

PDVSA

ALMACENAMIENTO TRATAMIENTO Y TRANSPORTE DE CRUDO
TRATAMIENTO Y LABORATORIO
LABORATORIO DE PETROLEO
TIA JUANA

INFORME DE ENSAYO

Solicitado por: MANUEL ANTONIO PEREZ TRUJILLO
Fecha de Toma: 07/06/2013
Fecha de Análisis 07/06/2013

RESULTADO DE ANALISIS FISICOQUÍMICOS AGUA DEL LAGO 07/06/13

MUESTRA	CONTROL 07/06/13	DOSIS 20ml 07/06/13	DOSIS 40ml 07/06/13
FECHA DE TOMA	07/06/2013	07/06/2013	07/06/2013
FECHA DE ANALISIS	07/06/2013	07/06/2013	07/06/2013
Temp (°F)	78	78	78
pH	8,39	8,32	8,08
ALC.	47,51	43,11	43,73
Na+	877,15	820,79	607,70
Resistividad	3,05	3,26	4,14
Conductividad	NO DETECTADO	NO DETECTADO	NO DETECTADO
CO3=	2,56	NO DETECTADO	NO DETECTADO
HCO3-	55,38	50,01	53,37
Cl-	1564	1468,00	1154,00
Ca++	24,03	21,21	22,94
Mg++	76,00	72,51	75,86
DT	378,80	374,20	373,30
SiO2	9,90	8,23	9,67
SO4=	26,00	22,00	19,00
Fe	N / D	N / D	N / D
Sol. Susp	6,00	22,00	28,00
Indice de STIFF 140°F	0,05	-0,11	-0,26
Indice de STIFF 180°F	0,40	0,23	0,07
Indice de STIFF 200°F	0,59	0,43	0,26
Tendencia de STIFF 140 °F	INCRUSTANTE	NO INCRUSTANTE	NO INCRUSTANTE
Tendencia de STIFF 180 °F	INCRUSTANTE	INCRUSTANTE	INCRUSTANTE
Tendencia de STIFF 200 °F	INCRUSTANTE	INCRUSTANTE	INCRUSTANTE

Realizado por
NESTOR GRANDA

Validado por
NOLBERTO PIÑA LIDER DE LABORATORIO

Revisado por
GERARDO OSORIO

Aprobado por
GERARDO HERNANDEZ GTE. DE LAB. C.O

IMPRESO: 10/06/2013

REPÚBLICA BOLIVARIANA DE VENEZUELA
MINISTERIO DEL PODER POPULAR PARA LA DEFENSA
FUERZA ARMADA NACIONAL
GUARDIA NACIONAL BOLIVARIANA
DIRECCION DE OPERACIONES
LABORATORIO CENTRAL
LABORATORIO REGIONAL N°3
JEFATURA

Maracaibo, 21 de Junio de 2013
203° y 154°

No. CG-CO-LC-LR3-1291

DEL	CORONEL JEFE DEL LABORATORIO REGIONAL N° 3 DE LA GUARDIA NACIONAL BOLIVARIANA.
AL CDDNO.	FÉLIX OSORIO GUZMÁN. MINISTRO DEL PODER POPULAR PARA LA ALIMENTACIÓN
ASUNTO	INFORME DE ANÁLISIS MICROBIOLÓGICO.
REFERENCIA	OFICIO DE SOLICITUD NRO. 000516 DE FECHA 13MAY13.

Tengo el agrado de dirigirme a usted, en la oportunidad de acusar recibo a la comunicación citada en la referencia, en atención a la solicitud, le remito anexo al presente oficio, un (01) informe de análisis microbiológico y anexos fotostático , el cual consta de tres (03) folios útiles.

Comunicación y remisión que hago a usted, para su conocimiento y fines legales consiguientes.

Dios y Federación,

WAYME JOSÉ MARÍN OCANTO

WJMO/guerra.-
Anexo: lo indicado...

"CHÁVEZ VIVE...LA LUCHA SIGUE"
INDEPENDENCIA Y PATRIA SOCIALISTA...VIVIREMOS Y VENCEREMOS"

Dirección: final de la Av. Guajira con final de la Av. El Milagro, sector las peonías (CORE-3) Correo electrónico: laboratoriocriminalistico3@hotmail.com

REPÚBLICA BOLIVARIANA DE VENEZUELA
MINISTERIO DEL PODER POPULAR PARA LA DEFENSA
FUERZA ARMADA NACIONAL
GUARDIA NACIONAL BOLIVARIANA
DIRECCION DE OPERACIONES
LABORATORIO CENTRAL
LABORATORIO REGIONAL N° 3
DEPARTAMENTO DE BIOLOGIA

Maracaibo, 21 de Junio de 2013

203° y 154°

INFORME DE ANÁLISIS MICROBIOLOGÍCO

Quien suscribe, **TTE. MARLLING GUERRA MEDINA, C.I: 13.628.205,** Experto del Laboratorio Regional N° 3, quien fue asignada por el **CNEL. WAYME JOSE MARÍN OCANTO** Jefe del Laboratorio Regional N° 3, para colectar y realizar análisis microbiológico a la cantidad de cuatro (04) muestras de agua; colectadas de cuatro (04) tanques plásticos de color celeste con capacidad de setecientos litros (700 L) c/u los cuales fueron llenados con agua del Lago de Maracaibo localizados en la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia. Dicho muestreo se lleva a cabo con la finalidad de realizar pruebas pilotos a un producto natural, a base de extractos de frutas, que funciona como sanitizante, agregado a los tanques de agua para observar el comportamiento del mismo. Las muestras se colectaron dando cumplimiento a la solicitud emitida por el Ciudadano **FÉLIX OSORIO GUZMÁN, MINISTRO DEL PODER POPULAR PARA LA ALIMENTACIÓN,** mediante oficio **Nro. 000516 DE FECHA 13MAY13.** La Ciudadana **VIANNET SEREIDA GUILLÉN C.I.V- 15.286.996** Ingeniero Químico, Analista II Control de Calidad de mencionada procesadora, procedió a colectar las muestras en recipientes de vidrio previamente esterilizados, de color ámbar, cerrados con tapa de plástico de rosca, con capacidad de doscientos cincuenta mililitros (250ml) cada uno, las muestras se identificaron con los números del **"1 al 4"**, y se trasladaron al Laboratorio Regional N° 3 de la Guardia Nacional Bolivariana, para practicarles los análisis microbiológicos correspondientes.

DESCRIPCIÓN FÍSICA DE LAS MUESTRAS:

1. Muestra 1: Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 01,** y se anotaron los siguientes datos: "MUESTRA 1: COLECTADA DEL TANQUE IDENTIFICADO COMO PATRÓN DE FECHA 05JUN13. HORA: 10:35 AM".--

2. Muestra 2: Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 02** y se anotaron los siguientes datos: "MUESTRA 2: COLECTADA DEL TANQUE IDENTIFICADO COMO PATRÓN DE FECHA 06JUN13. HORA: 09:00 AM".--

3. Muestra 3.: Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 03** y se anotaron los siguientes datos: "MUESTRA 3: COLECTADA DEL TANQUE IDENTIFICADO COMO 20 ML DE FECHA 06JUN13. HORA: 09:05 AM".--

CONTINUACIÓN DEL INFORME DE ANÁLISIS MICROBIOLOGICO SEGÚN OFICIO DE SOLICITUD NRO Nro. 000516 DE FECHA 13MAY13.------------------

REPÚBLICA BOLIVARIANA DE VENEZUELA Ministerio del Poder Popular para la Defensa Guardia Nacional Bolivariana CENTRO DE OPERACIONES

4. Muestra 4: Liquido de apariencia turbia, con particulas en suspensión y particulas sedimentadas. El envase se identificó con el **Nro. 04** y se anotaron los siguientes datos; "MUESTRA 4: COLECTADA DEL TANQUE IDENTIFICADO COMO 40 ML DE FECHA 06JUN13. HORA: 09:10 PM".---

Se procedió a la realizar los análisis microbiológicos en la siguiente secuencia analítica:

ANÁLISIS MICROBIOLÓGICOS:

1. Ensayo de Orientación para Coliformes: Determinado por la Técnica de Tubos Múltiples de Fermentación con dilución, y son reportados en términos de Número Más Probable (NMP/100ml) de organismos presentes.--

2. Ensayo de Certeza para Coliformes totales: Determinado por la Técnica de Tubos Múltiples de Fermentación con dilución, y son reportados en términos de Número Más Probable (NMP/100ml) de organismos presentes.---

RESULTADOS:

De los ensayos y análisis practicados a las muestras colectadas, se obtuvieron los siguientes resultados:

Tabla de resultados de los Análisis Microbiológicos del agua:

Muestras	Coliformes totales/en 100ml NMP
01	> 24.000
02	> 24.000
03	> 24.000
04	> 24.000
* Límite Máximo	< 5.000

(*) De acuerdo a lo establecido en el Decreto Presidencial número 883 publicado en la Gaceta Oficial de la República de Venezuela N° 5.021 del 18DIC95. **Artículo 10** (De las descargas a cuerpos de agua). Números más probable (NMP) de organismos Coliformes totales no mayor de 1000 por cada 100 ml, en el 90% de una serie de muestras consecutivas y en ningún caso será superior a 5000 por cada 100 ml. --

CONCLUSIONES:

En base al estudio y evaluación de los resultados obtenidos en los análisis microbiológicos realizados a cuatro (04) muestras liquidas identificadas con los Nros. 01 al 04, colectadas de seis (06) tanques llenados con agua del Lago de Maracaibo localizados en la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia, dando cumplimiento a las instrucciones del Ciudadano **FÉLIX OSORIO GUZMÁN, MINISTRO**

CONTINUACIÓN DEL INFORME DE ANÁLISIS MICROBIOLÓGICO SEGÚN OFICIO DE SOLICITUD NRO Nro. 000516 DE FECHA 13MAY13.----------

4. Muestra 4: Líquido de apariencia turbia, con partículas en suspensión y partículas sedimentadas. El envase se identificó con el **Nro. 04** y se anotaron los siguientes datos: "MUESTRA 4: COLECTADA DEL TANQUE IDENTIFICADO COMO 40 ML DE FECHA 06JUN13. HORA: 09:10 PM".----------

Se procedió a la realizar los análisis microbiológicos en la siguiente secuencia analítica:

ANÁLISIS MICROBIOLÓGICOS:

1. Ensayo de Orientación para Coliformes: Determinado por la Técnica de Tubos Múltiples de Fermentación con dilución, y son reportados en términos de Número Más Probable (NMP/100ml) de organismos presentes.----------

2. Ensayo de Certeza para Coliformes totales: Determinado por la Técnica de Tubos Múltiples de Fermentación con dilución, y son reportados en términos de Número Más Probable (NMP/100ml) de organismos presentes.----------

RESULTADOS:

De los ensayos y análisis practicados a las muestras colectadas, se obtuvieron los siguientes resultados:

Tabla de resultados de los Análisis Microbiológicos del agua:

Muestras	Coliformes totales/en 100ml NMP
01	> 24.000
02	> 24.000
03	> 24.000
04	> 24.000
* Límite Máximo	< 5.000

(*) De acuerdo a lo establecido en el Decreto Presidencial número 883 publicado en la Gaceta Oficial de la República de Venezuela N° 5.021 del 18DIC95. **Artículo 10** (De las descargas a cuerpos de agua). Números más probable (NMP) de organismos Coliformes totales no mayor de 1000 por cada 100 ml, en el 90% de una serie de muestras consecutivas y en ningún caso será superior a 5000 por cada 100 ml. ----------

CONCLUSIONES:

En base al estudio y evaluación de los resultados obtenidos en los análisis microbiológicos realizados a cuatro (04) muestras líquidas identificadas con los Nros. 01 al 04, colectadas de seis (06) tanques llenados con agua del Lago de Maracaibo localizados en la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia, dando cumplimiento a las instrucciones del Ciudadano **FÉLIX OSORIO GUZMÁN, MINISTRO**

CONTINUACIÓN DEL INFORME DE ANÁLISIS MICROBIOLOGICO SEGUN OFICIO DE SOLICITUD NRO Nro. 000516 DE FECHA 13MAY13.----------------

4. Muestra 4: Liquido de apariencia turbia, con particulas en suspension y particulas sedimentadas. El envase se identificó con el **Nro. 04** y se anotaron los siguientes datos: "MUESTRA 4: COLECTADA DEL TANQUE IDENTIFICADO COMO 40 ML DE FECHA 06JUN13. HORA: 09:10 PM".--

Se procedió a la realizar los análisis microbiológicos en la siguiente secuencia analítica:

ANÁLISIS MICROBIOLÓGICOS:

1. Ensayo de Orientación para Coliformes: Determinado por la Técnica de Tubos Múltiples de Fermentación con dilución, y son reportados en términos de Número Más Probable (NMP/100ml) de organismos presentes.---

2. Ensayo de Certeza para Coliformes totales: Determinado por la Técnica de Tubos Múltiples de Fermentación con dilución, y son reportados en términos de Número Más Probable (NMP/100ml) de organismos presentes.---

RESULTADOS:

De los ensayos y análisis practicados a las muestras colectadas, se obtuvieron los siguientes resultados:

Tabla de resultados de los Análisis Microbiológicos del agua:

Muestras	Coliformes totales/en 100ml NMP
01	> 24.000
02	> 24.000
03	> 24.000
04	> 24.000
* Límite Máximo	< 5.000

(*) De acuerdo a lo establecido en el Decreto Presidencial número 883 publicado en la Gaceta Oficial de la República de Venezuela N° 5.021 del 18DIC95. **Artículo 10** (De las descargas a cuerpos de agua). Números más probable (NMP) de organismos Coliformes totales no mayor de 1000 por cada 100 ml, en el 90% de una serie de muestras consecutivas y en ningún caso será superior a 5000 por cada 100 ml. ---

CONCLUSIONES:

En base al estudio y evaluación de los resultados obtenidos en los análisis microbiológicos realizados a cuatro (04) muestras liquidas identificadas con los Nros. 01 al 04, colectadas de seis (06) tanques llenados con agua del Lago de Maracaibo localizados en la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia, dando cumplimiento a las instrucciones del Ciudadano **FÉLIX OSORIO GUZMÁN, MINISTRO**

CONTINUACIÓN DEL INFORME DE ANÁLISIS MICROBIOLOGÍCO SEGÚN OFICIO DE SOLICITUD NRO Nro. 000516 DE FECHA 13MAY13.----------------

DEL PODER POPULAR PARA LA ALIMENTACIÓN, mediante oficio Nro. 000516 DE FECHA 13MAY13, se concluye que:

A. Desde el punto de vista microbiológico, las muestras de agua, identificadas con los números **"01 al 04"**, colectadas de los seis (06) tanques de la Procesadora de pescado "LA CAMARONERA", ubicado en el Municipio Santa Rita del Estado Zulia, ***no cumplen*** con los limites máximos permitidos y exigidos en el Articulo 10 (De las descargas a cuerpos de agua), de acuerdo a la clasificación establecida por las Normas Técnicas de Clasificación y Control de la Calidad de los Cuerpos de Agua y Vertidos o Efluentes Líquidos, del Decreto Presidencial número 883, publicado en la Gaceta Oficial de la República de Venezuela Nro. 5.021 del 18DIC95.--

B. Con lo antes expuesto, doy por concluido la actuación pericial y cumplimos con consignar el presente Informe de análisis microbiológico el cual consta de tres (03) folios útiles. ----------

Experto,

MARLLING GUERRA MEDINA
C.I: 13.628.205
TTE. LCDA. EN BIOLIOGÍA

Research water optimalisation Nicaragua 2020

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CENTRO PARA LA INVESTIGACIÓN EN RECURSOS ACUÁTICOS DE NICARAGUA
CIRA/UNAN-MANAGUA

PRELIMINARY REPORT

Management of the Laguna de Tiscapa Protected Area: Assessment of water and sediment quality

Prepared by CIRA / UNAN-Managua for:

Mayor of Managua

General Director of Environment and Urbanism

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1. Introduction

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The natural ecosystems located in urban areas such as the Tiscapa lagoon receive a great impact from the basin due to wastes of organic and inorganic compounds, some of natural origin (allochthonous organic matter and sediments) and others of anthropogenic origin (fertilizers and pesticides) . These compounds can be diluted or degraded naturally, however, when the entry of pollution occurs in large quantities or in high concentrations, it does not allow the internal metabolism of the lake to self-purify it and a process of environmental deterioration begins.

According to studies carried out by MARENA and CIRA / UNAN-Managua since 1989, the main problems of Tiscapa have been identified as the contribution of sediments from its entire drainage area (32.8 km^2) and its use as a receiving body of water. rainwater and domestic waste from the channels of San Isidro de la Cruz Verde (since 1958), Jocote Dulce and los Duartes (1980), which cross more than 20 neighborhoods and are drained through an artificial channel or channel. This has caused its waters to be contaminated from a bacteriological point of view and are not suitable for human consumption or recreation.

That is why since 1983 studies have been carried out to know the physical-chemical and biological conditions of Tiscapa , and to monitor its response to the high load of nutrients and pollutants it receives, generally showing green colored waters, as a product of massive growth. microalgae (blooms), which can be toxic, depending on the dominant species. In this context, it is also important to study the quality of the sediments since they can accumulate potentially toxic metals that reach the lake environment, which can be mobilized or released into the water column due to changes in their redox condition . There have also been many recovery efforts such as manual cleaning and biological treatment, generating positive results in the short term.

In this context, the Managua Mayor's Office, concerned about the environmental situation caused by the drainage of waste and sediments into the Tiscapa lagoon, has financed this study on the quality of the water and sediments in the bottom to know their impact on the lagoon. . Currently, the potential uses of the water of the Lagoon are limited due to contamination by domestic and industrial wastewater, plastic waste, garbage and by sediments originated by anthropogenic activities such as agriculture in the upper part of the micro-basin that directly reach through the channels to the lagoon due to rainfall in the rainy season. This component of the study was carried out to know the impact of the erosion rates, sediment carryover of the basin on the distribution of the particle size, organic matter content and total phosphorus.

That is why it is extremely important to know the biological physical, chemical and ecosystem that determine its quality for different potential uses of the water and the sediment s .

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2. goals

2.1. General objective .

Assess the environmental quality of water and sediments laguna of Tiscapa .

2.2. Specific objectives

a. Evaluate the environmental quality and current trophic state of the Tiscapa lagoon based on the physicochemical , microbiological and hydrobiological parameters of the water of the Tiscapa Lagoon

b. Characterization of the quality of the sediments to contribute to the environmental management of the Tiscapa Lagoon .

3. Justification of the Project or

The purpose of the project is to execute the sampling plan for the water and sediment quality parameters of the Tiscapa lagoon , based on what is established in the technical sheet. The preparation of a report that incorporates the scientific-technical elements that define the quality of the water and sediment in the Tiscapa lagoon in order to verify the efficiency of the technological equipment installed and the bio-stimulating treatment of the water. In compliance with the plan of activities programmed in the project , three sampling campaigns have been carried out in the lagoon, carried out by the technical staff of CIRA / UNAN-Managua. The first campaign took place in June , the second in July and the third in August , representative months of the season rainy.

4. Study area

The Laguna de Tiscapa nature reserve is located in the center of the City of Managua 2 km south of the shore of Lake Xolotlán and is part of the central system of the hydrogeological unit of the Las Sierras aquifer. Hydrographically it belongs to Sub-basin II of the southern sub-basin of Lake Xolotlán, with an approximate area of 33.7 km^2. The water mirror covers an area of approximately 0.14 km^2 and with an average depth that fluctuates seasonally between 31 and 42 m (Fig. 1).

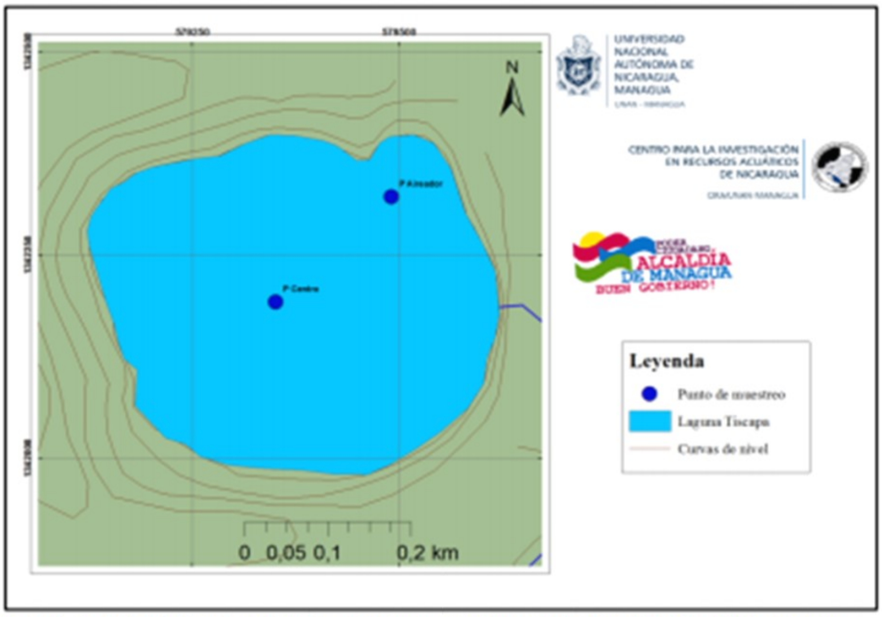

Figure 1 . Location of the Tiscapa lagoon and the two sampling points selected for the 2020 monitoring.

5. Description of the work carried out

5.1. Sampling

Samples were taken at two points in the lagoon; a point at the center of the lagoon named "Punto Centro" and the other located where the aerator is, "Punto Aireador". In the center point of water samples they were taken for physicochemical analysis, nutrients, bacteriological and hidrobiológi co phytoplankton and zooplankton) (Figure 1).

5.2. On-site measurements

Measurements were made at the center point of the Tiscapa lagoon , in three depth levels; at 0, 20 and 39 m respectively of pH, temperature , conductivity , dissolved oxygen and redox potential , to measure these variables is used rum laptops field and samples for measuring dissolved oxygen were taken in bottles Winkler

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5.3. Collection and analysis of samples for parameters determine f i sico chemical s , bacteriological and hidrobiol ógicos

Is colect arons m obtain water samples in the point ce nter of the lagoon at three depths ; on the surface (0, 20 and 39 m) , for f analysis i physicochemical , bacteriological and hidrobiológicos. The parameters chemical consisted nutrients (ammonium, total nitrogen , total phosphorus) , hydrogen sulfide , to the total lcalinidad, biological oxygen demand, chemical oxygen demand and carbon dioxide . Likewise, samples were taken for Thermotolerant Coliforms and for hydrobiological characterization samples were taken for phytoplankton and zooplankton. In Table 1 , the methodologies used for the collection, transport and preservation of the samples are shown .

T abla 1 . Parameters, type of containers, mode of transport and preservation of the samples.

Parameters	Container type	Transportation and preservation
Fí physicochemical	Polyethylene containers 4 liters of capacity	Portable cooler with ice
Nutrients	1000 ml one liter polyethylene jars	Preserved with 1 ml of concentrated H_2SO_4
Hydrogen sulfide	100 ml glass jars	4 drops of 2N zinc acetate Portable cooler with ice
Biochemical Oxygen Demand	Plastic Container 1000 ml	Portable cooler with ice.
Chemical Oxygen Demand	Bottle glass 100 ml	Add H_2SO_4 at pH <2, Cooler with ice
Thermotolerant C oliforms	1000 ml sterilized plastic containers	Portable cooler with ice
Phytoplankton	250 ml plastic containers	Portable cooler with ice
Zooplankton	250 ml plastic containers	90% alcohol

In Table 2 , the methodologies are shown to NALI ticas used and n the realizació n samples for analysis physicochemical , microbiological and hidrobiológicos .

Table 2 . Analysis methodology performed on the water samples collected in Tiscapa .

Analysis	Methods	Unit	Limit and / or range quantifies tion
pH	4500.HB [1]	PH units	0.10 to 14.00
Electric conductivity	2510.B [1]	$\mu S.cm^{-1}$	1.00 to 100.00.00
Total alkalinity	2320.B [1]	$mg.l^{-1}$	1.86
Free carbon dioxide	4500-CO_2,C [1]	$mg.l^{-1}$	*
Dissolved oxygen	4500-O_2,C [1]	$mg.l^{-1}$	0.20 to 25.00
Biochemistry demand Oxi geno	5210-B [1]	$mg.l^{-1}$	1.00
Chemical demand Oxi geno	5220-C [1]	$mg.l^{-1}$	10.00
Total Phosphorus	4500-PBE [1]	$mg.l^{-1}$	0.48
Total Nitrogen	Second derivative [2]	$mg.l^{-1}$	0.333
Ammonium	4500.NH_3.F [1]	$mg.l^{-1}$	0.021
Sulfide hydro geno	Fonselius [3]	$mg.l^{-1}$	0.04
Total Coliforms	9221-B [1]	MPN / 100 ml	<1.8

References :

merican Public Health Association (APHA) (2012). Standard Methods for the Examination of Water and Wastewater 22 -Edition Washington: APHA
2 Crumpton, WG, TM Isenhart & PD Mitchell. (1992). Nitrate an organic N analysis with second-derivate spectroscopy. Limnology & Oceanography 37: 907-913.
3 Grasshoff K., Kremling K. & Ehrhardt M. *Methods of Seawater Analysis*. (1999). Federal Republic Germany. Third Edition. Wiley-Vch.

5.4. Sediment sample collection and analysis

A surface sediment sample was collected at 800 m from the Aerator, using a Van Veen dredge to perform analysis of moisture content, granulometry, organic matter and total phosphorus (Table 3). The samples were collected and transported following the Standard Operating Procedures (SOP) of the Environmental Radiochemistry laboratory.

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Table 3 . Physical and chemical methods in sediments.

Parameter	Method	Limit or range of detection
Granulometry	Austrian Standard L - 1061 - 88 [2]	0.% 1 00 %
Organic material	MO Stainless [1]	0.1% to 13.13 %
Total phosphorus	Ascorbic Acid [1]	0.01 mg.g [-1]
C ontent moisture	Gravimetric [1]	0.1% to 99%

[1]Nom-021-SEMARNAT-200. Official Mexican Standard. That establishes the specifications of fertility, salinity and soil classification. Studies, sampling and analysis.

6. Results and D iscussion

6 .1 Physicochemical Parameters

6 .1.1 Distribution of temperature and dissolved oxygen (DO)

The temperature as f abiotic actor also plays a key role in the functioning of ecosystems regular or affect some abiotic factors such as solubility of nutrients and gases and other physicochemical properties such as pH, redox potential and density . The temperature ranged between 27.6 and 31.6 ° C with a mean value of 29.6 ° C (Fig. 2). The recorded temperature values show that there is no thermal stratification since the water in the Tiscapa lagoon is in homogeneous temperature conditions. In the only month that a greater difference in temperature between the surface and the bottom was observed , it was in the month of August, observing a decrease of 3.4 ° C, it is very likely that this is due to the high temperatures that occur. produced for this month, as a result of the heatwave period that is manifested in the national territory.

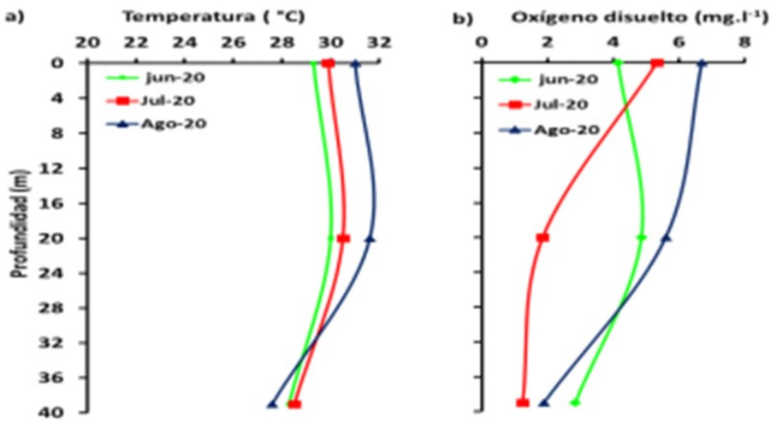

Figure 2 . Temperature distribution in the center point of the Tiscapa lagoon in the 2020 rainy period (between June and August).

Dissolved oxygen is the most important parameter to estimate the state and quality of water in a lake, since it is essential for the metabolism of aquatic organisms that present aerobic respiration (Wetzel , 1981). On the other hand, the concentrations of dissolved oxygen ranged between 1.24 and 6.70 and with a mean value of 3.83 mg.l $^{-1}$, with dissolved oxygen being found in the entire water column, no anoxia was observed in the timing of these samplings.

In the month of June it showed an almost homogeneous behavior, with low concentrations of oxygen in the entire water column, below the minimum concentration of 5 mg.l $^{-1}$ necessary for the development of aquatic life, according to the Council's guidelines. Canadian Ministers of the Environment (CCME, 2008). For July a slight increase in oxygen is observed especially in the lagoon surface and August this increase prevailed to 20 m depth slightly above of the minimum concentration of 5 mg.l $^{-1}$ necessary for the development of aquatic life of the above mentioned standard (Figure 3) . It should be mentioned that the lagoon exhibits an obvious and relatively respects improvement to l as oxygen concentrations reported in the sampling period, especially in August, as in previous sampling periods have been detected very low concentrations of oxygen on the surface and complete anoxia from 4-5 m depth (ALMA, CIRA / UNAN-MANAGUA, 2019) , which could be due to the treatment applied to the Lagoon with the product called PoCo and / or the dilution effect due to the rainy season when the volume of the bucket increases.

6 .1.2 pH distribution

The pH exhibited values that ranged between 6.73 and 8.24 units in the center of the lagoon in the studied period , observing slightly alkaline concentrations on the surface and slightly acidic at the bottom. This decrease in pH in the tropholytic zone (bottom) is produced by the generation of CO_2 due to respiration, this also occurs because the decomposition and respiration processes result in the release of CO_2, consequently the formation of acid. carbonic and hydrogen ions . Several metabolic processes can generate hydrogen ions and with this, contribute to lower the pH of the medium, among these are the biological oxidation processes in general, nitrification processes, the oxidation of sulfides, all these processes cause an increase in CO_2 and a decrease in pH. These results are within the pH range recommended by the Canadian Standards for the protection of aquatic life (6.5 to 9.0) and for recreational use (total body contact) of 6.5 to 8.5 (CCME, 2008) (Fig. 3) .

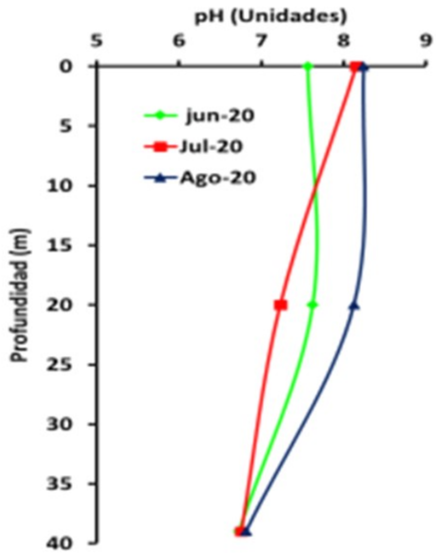

Figure 3 . Distribution of the pH in the center point of the Tiscapa lagoon in the rainy season (between June and August 2020).

6 .1. 3 Distribution of free Carbon Dioxide (CO_2)

Free carbon dioxide (CO_2) is the second most important gas present in water, it is caused by the decomposition of organic matter, by the respiration of animals and plants, by the excretion products of these organisms. and by rainwater. Free CO_2 registered values from 0 to 50.3 mg.l^{-1} with a general average of 11.6 mg.l^{-1}. In Figure 4, the behavior of free CO_2 throughout the study is shown . On the other hand, and n the surface of the l aguna, p ara June were recorded very low concentrations of CO_2 free and absence total months of July and August due to slightly values basic pH , as a result of the assimilation of CO_2 by phytoplankton . Also, a noticeable increase in the concentrations of free CO_2 was observed at the bottom of the lagoon, especially in the month of August, with the highest concentration reported in the sampling period (50.3 mg.l^{-1}) making evident the high decomposition of organic matter found in this area of the hypolimnion .

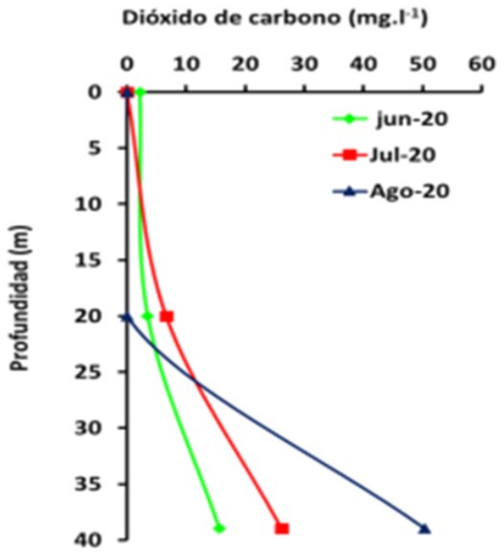

Figure 4 . Distribution of free carbon dioxide concentrations at the Center point at different depths in the Tiscapa lagoon .

6 .1. 4 Distribution of electrical conductivity

The conductivity ranged between 2 42.8 and 408.1 µS.cm -1 (Fig. 5) , with a mean value of 282.2 . µS.cm -1, observing the highest concentrations at the bottom of the lagoon, this behavior is considered normal due to the series of biologically mediated redox processes such as the degradation of organic matter (carbonaceous and nitrogenous) and the reduction of sulphates , iron and manganese that result in a net increase in alkalinity and dissolved metals in hypolimnetic waters (Wetzel , 2001). Conductivity generally increases progressively from the surface to the bottom in bodies of water (Rodier , 2009).

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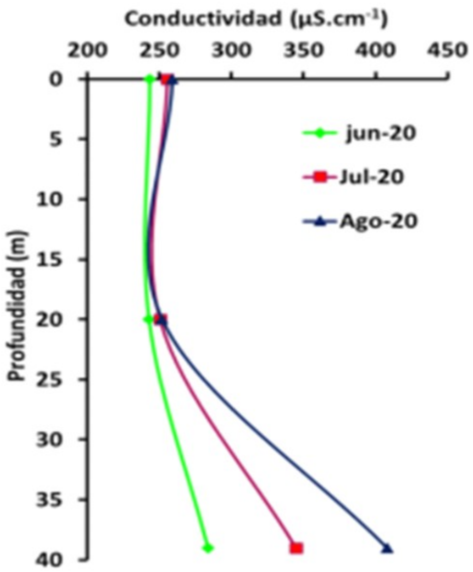

Figure 5 . Vertical distribution of electrical conductivity at the center point of the Tiscapa lagoon in the sampling period (between June and August 20-20)

According to Rodier (2009), the waters of the Tiscapa lagoon are classified as medium mineralization waters (200 µS.cm $^{-1}$ - 333 µS.cm $^{-1}$) and only the bottom point of the month of August (408, 1 µS.cm $^{-1}$) would be classified as water with marked medium mineralization (333 µS.cm $^{-1}$ - 666 µS.cm $^{-1}$) (Table 4).

Table 4 . Classification of the mineralization of waters by means of their conductivity according to Rodier (2009)

Electric conductivity	Classification
<100 µS.cm $^{-1}$	Very weak mineralization
100 - 200 µS.cm $^{-1}$	Weak mineralization
200 - 333 µS.cm $^{-1}$	Medium mineralization
333 - 666 µS.cm $^{-1}$	Accentuated average mineralization
666 - 1000 µS.cm $^{-1}$	Major mineralization
> 1000 µS.cm $^{-1}$	High mineralization

6.1.5 Total alkalinity

The alkalinity of the Tiscapa lagoon ranged between 91 and 1 73 mg.l ⁻¹ with a mean value of 113.3 mg.l ⁻¹. Naturally, alkalinity is generated as water makes contact with the soil and dissolves rocks that contain calcium carbonate, such as calcite or limestone. Alkalinity is mainly due to carbonate, bicarbonate and hydroxide ions and represents the capacity of an aquatic ecosystem to neutralize (buffer) the acids that have been added to it (Esteves, 1988) (Fig. 6) .

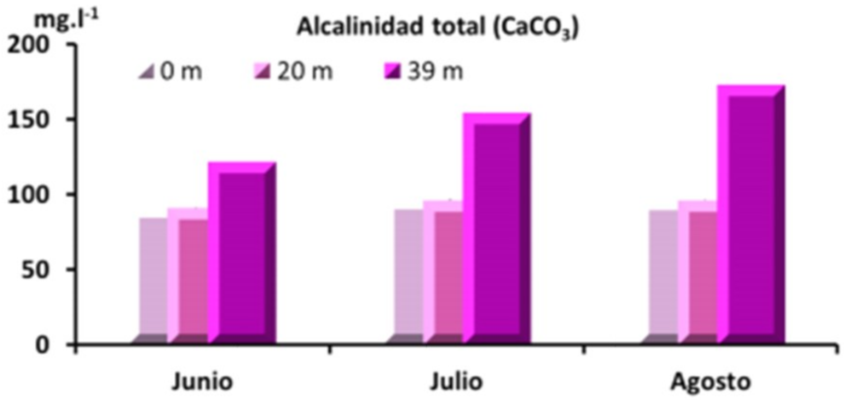

Figure 6 . Distribution of total alkalinity at the different depths studied in the Tiscapa lagoon (between June and August 2020) .

Alkalinity behavior was heterogeneous, observing low concentrations in surface and part medi to and higher in the bottom of the lagoon . According to these results, the Tiscapa lagoon presents characteristics of medium alkalinity when compared with the ranges observed in Table 5 , which indicates a medium buffering capacity to neutralize acids, which is attributed to bicarbonate ions. It is important to note that in the month of August (bottom) the lagoon presented a characteristic of high alkalinity. This characteristic is essential to protect the water from drastic changes in pH, since the lagoon is exposed to the influence of rainwater and domestic water that are conducted by the river, as well as sediment, all kinds of garbage and pollution.

Table 5 . Ranges of alkalinity in natural waters.

Rank	Alkalinity as mg l^{-1} $CaCO_3$
LOW	<75
HALF	75 - 150
HIGH	> 150

Taken from http://www.uprm.edu/biology/profs/massol/manual/p2-alcalinidad.pdf

6 .1.6 Biochemical oxygen demand (BOD_5) and Chemical oxygen demand (COD).

The BOD_5 is a measure of the amount of oxygen required by microorganisms to degrade and stabilize the organic matter in a water sample, through a heterogeneous microbial population and the COD is a measure of the amount of oxygen consumed that represents the content of organic matter oxidizable by dichromate in acidic solution of the sample and is constituted by biodegradable compounds and those that are more difficult to degrade due to their chemical composition (refractory) (Sawyer, 2001).

The BOD_5 concentrations for the rainy season ranged between 2.7 and 7.4 $mg.l^{-1}$ with an average of 5.3 $mg.l^{-1}$ (Fig. 7 a). On the other hand, the COD concentration for the studied sampling period ranged between values of 13.7 and 44.0 $mg.l^{-1}$ with an average of 21.1 $mg.l^{-1}$ (Fig. 7 b) .

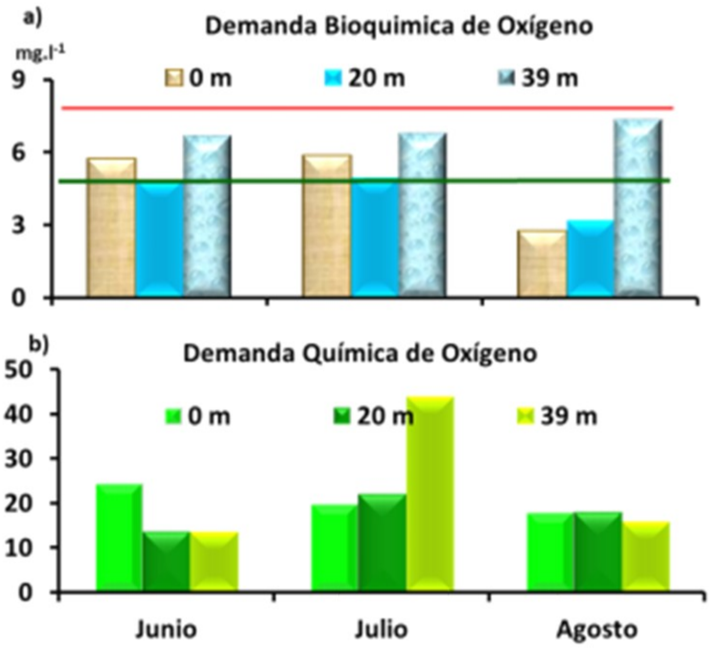

Figure 7 . Distribution of the concentrations of Biochemical Oxygen Demand (a) and Chemical Oxygen Demand (b) at the Center point at different depths in the Tiscapa lagoon .

Taking as reference the classification proposed by Ramírez & Viña (1998) to inquire about the situation of a body of water according to its BOD_5 (Table 6), the Tiscapa lagoon could be classified as a body of water of doubtful quality on the surface and middle part of the lagoon and of abnormal quality in the bottom of the same, that is to say that there is a high demand of oxygen by the organisms that are in this stratum of the lagoon. Also Custodio & Llamas (2001) considers that a body of water with a BOD_5 value greater than 6 mg.l^{-1} is suspected of contamination. However, it is necessary to mention that after the additions of the product called PoCo since June, both BOD and COD have decreased concentrations when comparing this sampling with the sampling of August 2018, observing an evident improvement in the month August, on the surface with a concentration of 2.72 mg.l^{-1} classifying the Lagoon as waters of acceptable quality and in the middle part (3.16 mg.l^{-1}) very close to acceptability.

Table 6 . Situation of the body of water according to its BOD_5. Ramírez & Viña (1998)

BOD concentration , mg.l^{-1}	Situation of the body of water
BOD_5 <1	Normal
1 <BOD_5 <3	Acceptable
3 <BOD_5 <6	Doubtful
BOD_5 > 6	Abnormal

The basic difference between COD and BOD_5 lies in the fact that there are more compounds that are chemically oxidized than biologically. Therefore, the COD values will be reasonably higher than those of BOD_5, to such an extent that a direct relationship between both parameters can be established (Table 7) (Sawyer, 2001).

Table 7 . Relationship between BOD_5 and COD parameters

Relationship	Little biodegradable	Biodegradable	Very biodegradable
BOD_5/ COD	<0.2	0.2 - 0.4	> 0.4

Finally, the BOD_5/ COD ratio was calculated for the Tiscapa lagoon in order to know the predominant type of organic matter, that is, if the compounds present in the water are biodegradable or not very biodegradable (recalcitrant), the results obtained are shown in Table 8 . Therefore, the BOD_5/COD ratio obtained for the Tiscapa lagoon for the months under study indicates the dominance of organic matter in the biodegradable range in 77.7% and only 22.2% was characterized by being very biodegradable. These results are as expected, considering that the greatest impact on this body of water is the sediment and garbage that the channel carries .

Table 8 . BOD_5/ COD ratio

BOD_5/ COD	June	July	August
0 m	0.2	0.3	0.2
20 m	0.4	0.2	0.2
39 m	0.5	0.2	0.5

6 .1. 7 Distribution of total nitrogen and ammonia.

Nitrogen is present in aquatic environments in several forms: the inorganic forms of nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+), molecular nitrogen (N_2) and the organic forms of organic nitrogen dissolved (peptides, amines, purines and amino acids) and particulate organic nitrogen (bacteria, phytoplankton, zooplankton and debris) (Esteves, 1988) . Total nitrogen refers to the sum of all these mentioned nitrogen fractions that can be found in water (Fig. 8). For the period studied, the concentration of total nitrogen ranged between 0.49 and 12.19 $mg.l^{-1}$. With an average value of 3.68 $mg.l^{-1}$, the highest concentrations being found in the bottom of the lake.

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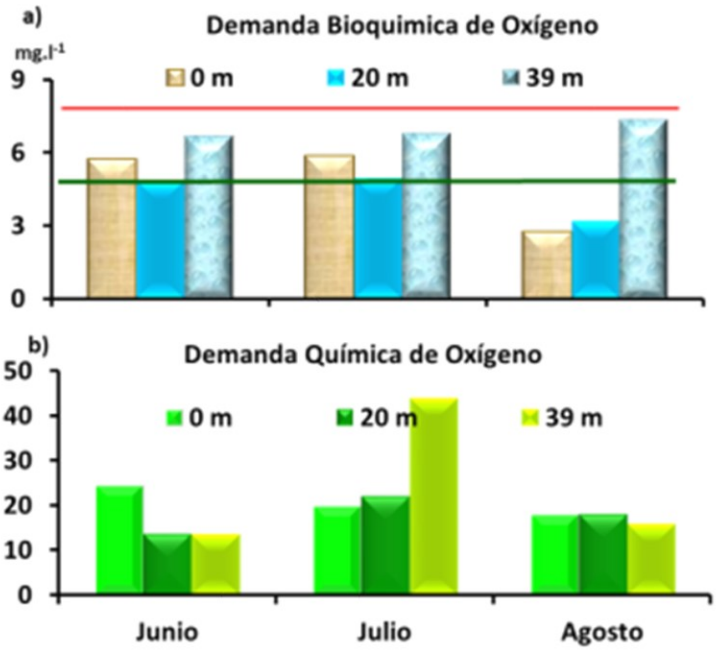

Figure 8 . Distribution of total nitrogen concentrations in the Tiscapa lagoon at the Centro point at different depths June-August 2020.

Ammonia nitrogen was the nitrogen species with the highest concentrations detected in the studied period, ranging between 0.04 and 9.28 mg.l-1 with an average of 2.98 mg.l-1 (Fig. 9), (It is worth mentioning that in June this variable was not requested). Very low concentrations between 0.04 and 0.09 mg.l -1 were found on the surface and middle part of the Lagoon . S ccording Wetzel , (1981) these concentrations are usually found in aquatic ecosystems, so it is very common not detect or found in very low concentrations in natural waters. It is very likely that this decrease in nitrogen in its ammoniacal form is due to the presence of oxygen in the water column, on the other hand, extremely high concentrations were detected in the deepest stratum of the l aguna, since this compound among others , is the final product of the decomposition of organic matter, the increase depending on the depth is due to the reductive processes that occur in the water column, which are conditioned by the levels of hypoxia that occurred in the ecosystem.

The production of ammonium (ammonification) is a process that is favored due to anoxic conditions as nitrification (production process nitrates) ceases due to the decrease of the redox potential (<400 mV) (Wetzel , 1981), it should be noted , that the redox potential values found in the Tiscapa lagoon are well below this value. Ammonium is also recognized for being the form of nitrogen that promotes high growth rates of cyanobacteria, which were identified as the dominant phytoplankton group in all the samplings carried out (Wetzel , 1981) . Ammonium has been reported as highly toxic (median lethal concentration, LC $_{50}$ = 0.50 mg.l $^{-1}$), especially for fish (Wetzel , 1981 & Esteves, 1988), a value that was exceeded at the bottom of the ecosystem.

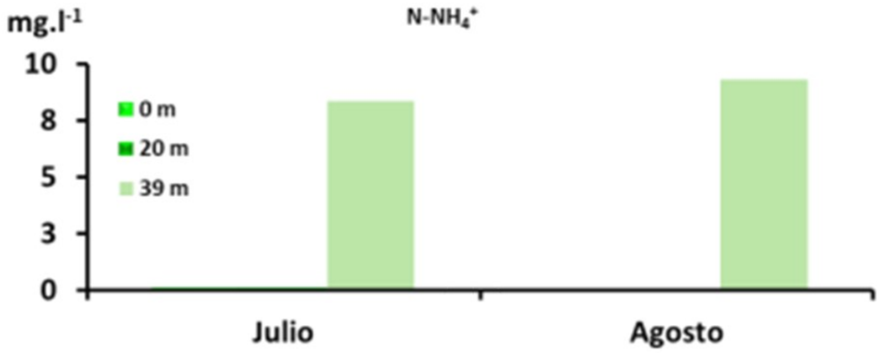

Figure 9 . Distribution of ammonia nitrogen concentrations in the Tiscapa lagoon . 2020.

These findings could be due to a positive effect of l treatment with bit applied to the lagoon Tiscapa , to the dilution effect due to the increase in the volume of water from the bucket at the time of sampling, it has n influenced improve water the same, since living organisms such as fish can live in ecosystems that have low concentrations of ammonia that do not exceed the lethal dose, as mentioned above.

6 .1.8 Distribution of total phosphorus.

Phosphorus plays an important role in the development of algae, it is capable of promoting their reproduction in the waters of lakes. According to Wetzel , (1981), the ecological interest of phosphorus stems from its important role in biological metabolism and its relative scarcity in the hydrosphere, compared to the natural abundance of the other nutritive and structural components of living matter (carbon, nitrogen, oxygen).

The total phosphorus concentrations ranged from 0.1 14 and 1.229 mg.l $^{-1}$ with an average value of 0.496 mg.l $^{-1}$ throughout the study period (Fig. 1 0). The total phosphorus distribution pattern observed in the lagoon indicates that the lowest concentrations were found at the surface (epilimnion) and at medium depth compared to the high concentrations at the bottom (hypolimnion). This pattern of phosphorus distribution could be attributed to the release of the phosphate ion from the sediments into the water column, a process that occurs more easily in conditions of low oxygen concentration , such as those found in the Tiscapa lagoon (Esteves, 1988).

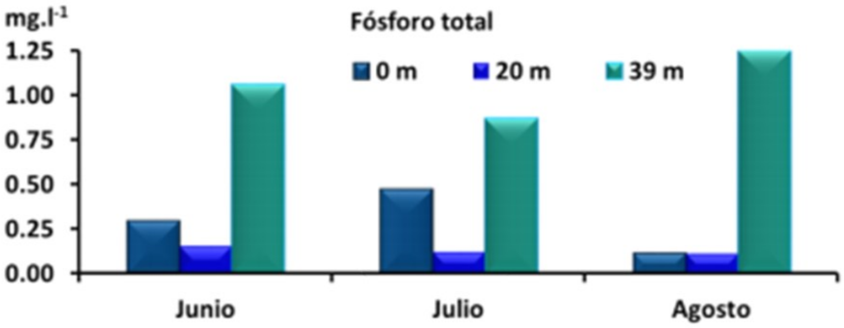

Figure 10 . Distribution of total phosphorus concentrations at the Center point at different depths.

All total phosphorus concentrations found in the Tiscapa lagoon exceed the recommended value of 0.025 $mg.l^{-1}$ by the United States Environmental Protection Agency (USEPA, 1986) to prevent the eutrophication process in lakes and dams. On the other hand, the European Environment Agency (EEA, 2000, 2005) considers that, in general, a total phosphorus concentration should not exceed the range 0.010 - 0.025 $mg.l^{-1}$PT to prevent eutrophication in rivers and lakes. , the concentrations in the Tiscapa lagoon exceed these concentrations.

It is important to mention that with this variable a slight improvement in the ecosystem is observed, with the addition of the PoCo treatment, since the concentrations in the middle part of the lagoon decreased, and in the month of August they decreased notably both on the surface and in the middle stratum. However, the concentrations in the background doubled the values in relation to the study carried out by ALMA, CIRA / UNAN-MANAGUA, 2019 , in this study a maximum value of 0.666 $mg.l^{-1}$ and for the present study a maximum of 1,249 $mg.l^{-1}$.

6 .1. 9 Trophic state

To assess the trophic status of a lagoon or reservoir, there are many methodologies, in this study to establish the trophic status of the Tiscapa lagoon the trophic status index for tropical lakes developed by Salas & Martino (2001) was used . However, the Carlson trophic status index modified by Toledo (IET_M) could not be performed , since orthophosphate was not taken , a variable considered of great importance because it is the form available for microalgae .

Eutrophication is the process of overproduction of algae and macrophytes in bodies of water that can cause problems in certain uses such as, among others: alteration of its organoleptic properties (smell, taste), decrease in oxygen content, accumulation of ammonia in the water column and resuspension of certain metals (Fe, Mn) from the sediment under anoxic conditions (Salas & Martino, 2001). Although it is a slow and continuous process, it can have a natural origin, it is characterized as a result of the contribution of nutrients carried by the runoff that erodes and washes the earth's surface, it can also be called natural aging of a lake. Today it is fundamentally cultural in nature, accelerated by the continuous supply of nutrients of anthropogenic origin. Artificial eutrophication is a dynamic process, in which profound qualitative and quantitative modifications occur in aquatic communities, in the physical and chemical conditions of the environment and in the production level of the system, which can be considered a form of contamination (Esteves, 1988).

The results obtained using the methodology & Martino Salas (2001) according to the probability curve of total phosphorus located to l aguna of Tiscapa in hypertrophic condition in a 93 % (Fig. 1 1). As can be seen, the mean value in this study was 496 mg P / cm ^{3} which when compared with the ALMA study , CIRA / UNAN-MANAGUA, 2019 of 374 mg P / cm ^{3} increased by 32.6%, also increasing in % the value of hypereutrophy going from 89% to 93%.

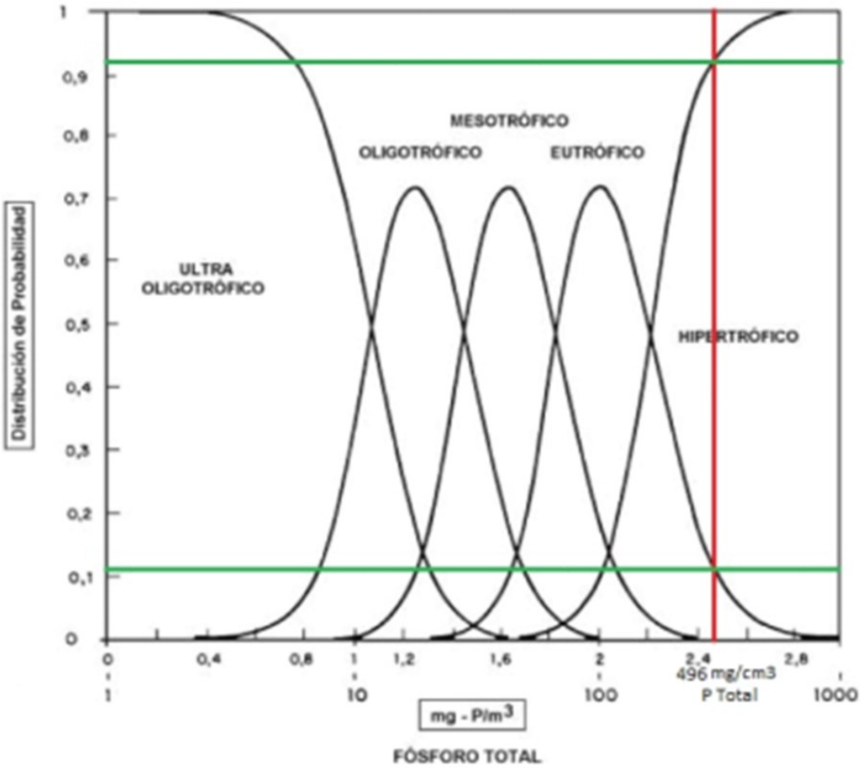

Figure 11 . Trophic level probability distribution of tropical warm lakes based on total phosphorus Salas & Martino (2001) with data from warm tropical lakes.

6.2 Microbiological contamination

The microbiological contaminations of surface water bodies are characterized through the detection of contamination indicator organisms for this study , Thermotolerant Coliforms were used , this group constitutes a group of heterogeneous bacteria with primarily intestinal habitat and is used as an indicator of fecal contamination. in water (Castro, 2002). In Fig ure 12 , the thermotolerant coliforms cuantifiación the indicated obtained and n these three months of study, l to greater concentration of these organisms reportaro n in June ranges 1.3 × October and 0, 2 × 10 NMP / 100 mL for the three depths (0, 20 and 39 m), decreasing the concentrations in the month of July in the depths of 0 and 39 meters in a range of 0.79×10^{2} and 1, 70 × 10^{2} NMP / 100 mL, respectively; However, for that same month, an increase in thermotolerant coliforms of 7.9 × 10 NMP / 100 mL was obtained at a depth of 20 meters. Finally, in the month of August, concentrations of 3.3 × 10 and 0.3 × 10 MPN / 100 mL were obtained for the same depths described above.

The results obtained with and without PoCo application and with and without aeration system, data provided by the Mayor's Office of Managua, (Molina, 2020) are variable for the three months of study, for the month of June a higher concentration of microorganisms, this is the reflection that the lagoon continues to receive anthropogenic input, dragging diverse material, be it plant and animal types, that are discharged into the lagoon through the channel, days prior to the sampling, PoCo was applied, in quantities of 114 - 150 liters per day not so for the sampling day and the aeration system was working before and during the sampling, for the month of July, despite the fact that PoCo was not applied for 12 days ago, nor was the sampling day, in addition to the fact that the system was not working. aeration results at 0 m were considerably low, followed by depth of 39 m; However, at a depth of (20 m) there was an increase in thermotolerant coliforms, generally coliform bacteria are found in greater abundance in the surface layer of water or in sediments (Ramos Ortega, Vidal, Vilardy Q, & Saavedra Díaz, 2008) low concentrations of coliforms termotoleranes surface could n be inflluenciado by a substance which inhibited the growth of the thermotolerant or coliforms by the incidence of the rays of solar influencing retention and survival of these organisms (Gonzalez, Paranhos, & Lutterbach, 2010).

For the month of August the behavior of the indicator organisms shows a decrease in relation to the month of June and at a depth of 20 m in July, since 2020-07-21 PoCo has been applied in variable amounts from 165 - 300 liters / day , even on the date the sampling was carried out, in addition to the aeration system being active, this reduction in organisms for this month could be explained in two ways, one is that the lagoon was not receiving anthropogenic contributions through the channel in quantities considerable and the other could be the result of the application of PoCo which is observed its effect in the decrease of thermotolerant coliforms.

In relation to the averages obtained from thermotolerant coliform organisms in the water column per month, they show that the Tiscapa lagoon exceeds those established by the Nicaraguan Obligatory Technical Regulation NTON 05-007 98 (Standard for the Classification of Water Resources, MIFIC / ONA, 1998) for water intended for agricultural uses for irrigation of vegetables, the permissible thermotolerant coliforms should be less than 100 NMP / 100 ml, for irrigation of any other type of crop and livestock use less than 1000 NMP / 100 ml. For spa waters with total human contact, it must be less than 200 NMP

/ 100 ml in 90% of a series of consecutive samples and less than 400 NMP / 100 ml in the remaining 10%, if the contact is partial, it must comply with the limit is 1000 MPN / 100 ml. Rules n icaragüenses match established by EPA (2012) US, that defined for the direct and prolonged contact, such as swimming 200 fecal bacteria (Thermotolerant) and 126 *Escherichia coli* per 100 ml, by Therefore, the concentrations detected in the Tiscapa lagoon exceeded the limits for the different uses.

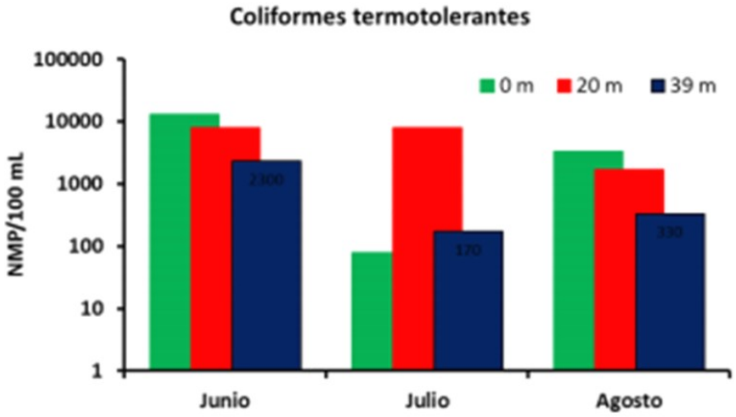

Figure 12 . Behavior of Thermotolerant Coliforms in the Center point of the Tiscapa Lagoon .

6.3 Phytoplankton and Zooplankton Structure

6.3.1 Phytoplankton Structure

Phytoplankton is the microscopic plant community present in aquatic systems, they present locomotion through flagella and hydrodynamic mechanisms that allow them to be distributed throughout the water column, however, most float freely in the surface layer. It is part of the producers of these ecosystems and they represent variants in their physiological requirements according to the group, maintaining tolerance levels to environmental conditions. (Wetzel RG, 1981) (Gonzáles, 1988) .

6.3.1.1 Community structure

Community Phytoplankton of Lagoon Tiscapa during the months of June, July and August 2020 was representad to four Algal divisions (Chlorophyta , Bacillariophyta , Cyanophyta , Cryptophyta) , a total of 29 identified taxa s (Appendix, Table 1) and (Annex , Photographs 1 (Cyan ophyta), 2 (Chlorophyta) and 3 (Bacillariophyta)) . The first three algal groups were the most representative Chlorophyta , Bacillariophyta and Cyanophyta with 48 , 35 and 14 %, respectively (Fig. 1 3).

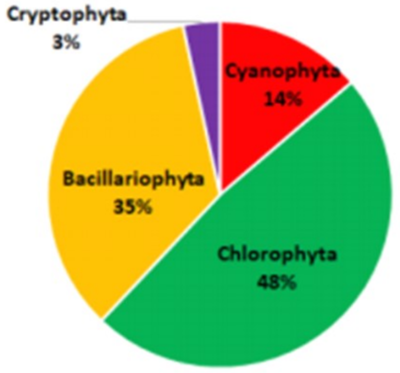

Figure 13. Contribution of the algal groups to the richness of species in the Tiscapa lagoon.

The group of Chlorophytas was the most representative during the three months of study in relation to the richness of species for the different depths (Fig. 14). The high representativeness of this group in freshwater ecosystems is due to their varied ecology and wide tolerance range, which allows them to colonize a wide spectrum of environmental conditions (Margalef R., 1983) and (Darley WM, 1987). Like the Chlorophyta, the Bacillariophyta are widely distributed in freshwater aquatic ecosystems, as well as brackish and salty ecosystems. These microorganisms have a siliceous structure that gives them a higher density, which affects the little presence of this group in the surface stratum (0 m) of the lagoon, reporting in the month of August at the average depth (20 m) the greater contribution to species richness.

The Cyanophyta, although they were presented with a low contribution to the richness (4 taxa), is an interest group since the presence and abundance of certain genera and / or species can cause serious difficulties in the management of water quality, thus as public health problems. According to (Roldan & Ramirez, 2008) they occur fundamentally when the environmental conditions deviate significantly from the usual conditions and it can be considered that any change in the nitrogen-phosphorus relationship ends up manifesting itself in an advance or a setback in the development of the blue green algae.

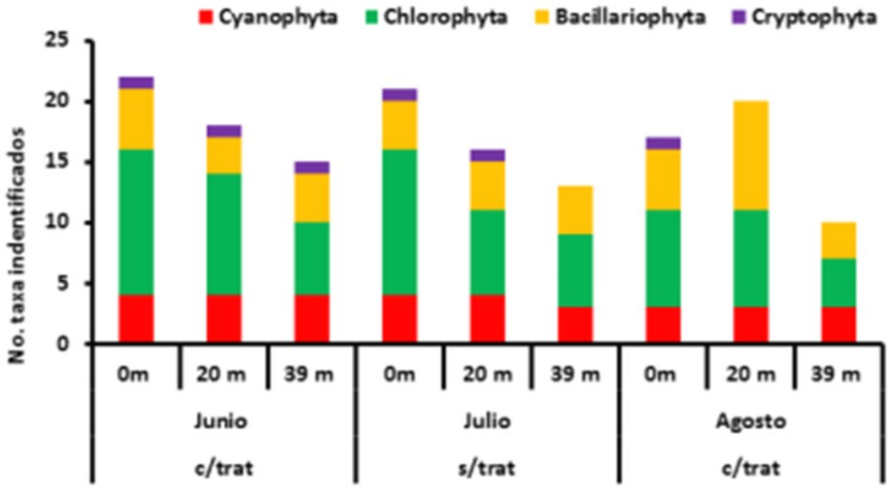

Figure 14 . Number of Taxes identified by taxonomic group at depths and months sampled at the center point of Laguna de Tiscapa .

In Table 9 , the most common taxons (n = 15) are mentioned , at the different depths and months sampled at the Center point . The Cyanophyta (*Merismopedia tenuissima* , *Microcystis aeruginosa* , *Oscillatoria limosa* and *Planktolyngbya* sp) presented a homogeneous distribution in the study period, on the other hand: Chlorophyta (8 taxa), Bacillariophyta (4 taxa) and Cryptophyta (1 taxa) , have the capacity adaptation and colonization in poorly c ampo lighting where the transparency values reported measured Secchi disk were low (June: 0.50; July 0.70, and August: 0,40).
Taking into account that not only blue-green algae are used as bioindicators of the trophic state, Bazán (2010) cited by (Sosa & Martínez, 2011) , mentions that the representation of fast-growing and high-renewal species such as *Scenedesmus* , *Monoraphidium* and *Chlorococcales* indicate a moderate to high degree of eutrophy .

Table 9 . Common taxa at different depths and months sampled .

Cyanophyta	**Cyanophyta**	**Cyanophyta**
* Merismopedia tenuissima	* Merismopedia tenuissima	* Merismopedia tenuissima
* Microcystis aeruginosa	* Microcystis aeruginosa	* Microcystis aeruginosa
* Silty oscillatoria	* Silty oscillatoria	* Silty oscillatoria
* Planktolyngbya sp	* Planktolyngbya sp	* Planktolyngbya sp
Chlorophyta	**Chlorophyta**	**Chlorophyta**
Actinastrum hantzschii	Actinastrum hantzschii	Closterium parvulum
Ankyra sp	Ankyra sp	* Coelastrum astroideum
Closterium limneticum	Closterium parvulum	* Coelastrum indicum
* Coelastrum astroideum	* Coelastrum astroideum	* Micractinium pusillum
* Coelastrum indicum	* Coelastrum indicum	* Monoraphidium contortum
Dictyosphaerium pulchellum	Dictyosphaerium pulchellum	* Oocystis lacustris
* Micractinium pusillum	* Micractinium pusillum	* Pandorina sp
* Monoraphidium contortum	* Monoraphidium contortum	* Scenedesmus armatus
* Oocystis lacustris	* Oocystis lacustris	* Scenedesmus semipulcher
* Pandorina sp	* Pandorina sp	**Bacillariophyta**
* Scenedesmus armatus	* Scenedesmus armatus	Achnanthes sp
* Scenedesmus semipulcher	* Scenedesmus semipulcher	* Aulacoseira granulata
Bacillariophyta	Scenedesmus sp	* Cyclotella meneghiniana
* Aulacoseira granulata	**Bacillariophyta**	Cymbella sp
* Cyclotella meneghiniana	Achnanthes sp	Fragilaria crotonensis
Cymbella sp	* Aulacoseira granulata	Gomphonema sp
* Nitzschia sp	* Cyclotella meneghiniana	Navicula sp
* Synedra ulna	* Nitzschia sp	* Nitzschia sp
Cryptophyta	* Synedra ulna	Pinnularia sp
* Cryptomonas sp	**Cryptophyta**	* Synedra ulna
	* Cryptomonas sp	**Cryptophyta**
		* Cryptomonas sp

* common taxa

6.3.1.2 Phytoplankton numerical abundance

Figure 15th behavior phytoplankton abundance in the illustrated p daub C entered and the contribution of large groups (Cyanophyta , Chlorophyta , Bacillariophyta and Cryptophyta) for months and depth is . The contribution of the algal groups to the total abundance at different depths was variable for the three months of sampling (June: CV% = 35-92, July: CV% = 47-139, August: CV% = 64-173) . The val or minimum and maximum reported at point C entered during the study period was: 4448595 Ind l⁻¹(June 39 m) and 106,146,231 Ind l⁻¹(July 0 m) . R esulting the highest total abundance in July (122 784 494 Ind l⁻¹), where the Cyanophytas turned out to be the most numerically representative group (Fig. 15b) .

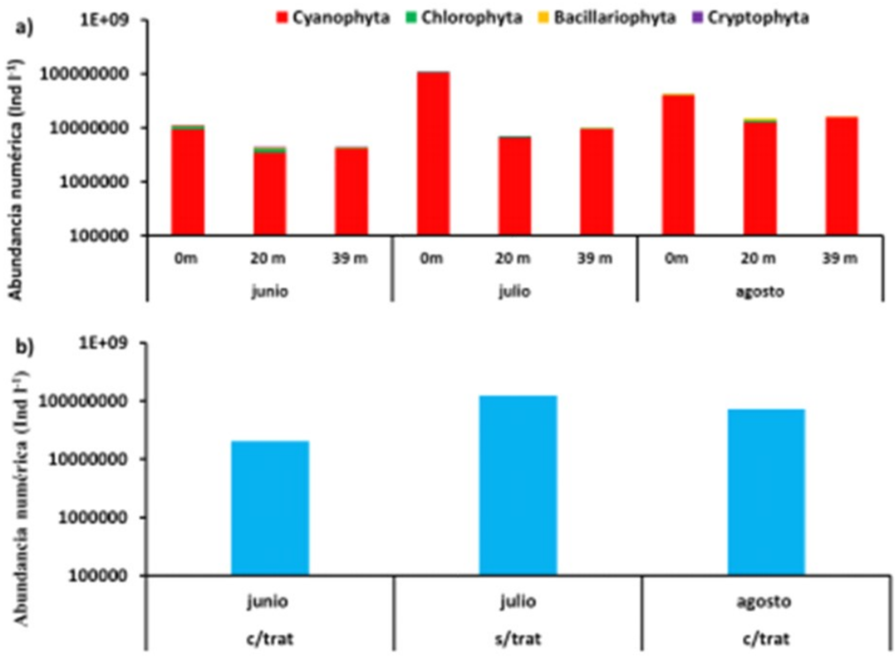

Figure 15 . a) Contribution of the phytoplankton groups to the total abundance in the different depths and months sampled in the Tiscapa lagoon and b) Total abundance of phytoplankton in the three months sampled (with and without PoCo application).

The Cyanophytas are an algal group having a wide range of tolerance to many factors including light, temperature, nutrients etc , so that adapts to difficult conditions. In this sense, (Roldan & Ramirez, 2008) , mention that these species, due to their wide range of tolerance, can be distributed in all the biotopes of the lake ecosystem (air-water interface, the entire water column, the sediment). So, in the studied environment (Center), the Cyanophyta were the dominant group for the three months at variable depths (0, 20, 39 m).

In the superficial stratum (0 m) in the three months sampled, the dominant species was *Microcystis aeruginosa* (Table 2). Certain species of cyanobacteria dominate over other phytoplankton organisms on the surface of the waters, due to a series of characteristics that provide them with certain competitive advantages, such as this species. Walsby & Hayes, 1989 cited in (Vela, et al., 2007) mention that they can regulate their buoyancy by means of gas vesicles to be placed in the water column at those depths where the availability of nutrients and light is adequate. The (UNESCO, 2009) , classifies *Microcystis aeruginosa* as a species that prefers still water environments, limnic systems of more than 5 m of stratification and that is characterized by producing different toxins: microcystins, lipopolysaccharides, cyanoginosins and volatile sulfur compounds.

From the average depth (20 m) to the bottom (39 m) the Cyanophyta continue to predominate, but with the presence of the genera *Planktolyngbya* and *Merismopedia*, the first associated with the production of saxitoxin, lyngbyatoxin -a, microcystin LR, and lipopolysaccharides and the genus *Merismopedia* linked to the last two toxins, (Chorus & Bartram, 1999) mentions its advantage in low light conditions, (due to the high population density in eutrophic waters that leads to their turbidity), so cyanobacteria can maintain a relatively higher growth rate than other phytoplankton organisms present.

It is important to note that this algal group predominates in waters where the pH is neutral or slightly basic (Roldan & Ramirez, 2008), and supports wide ranges of temperatures (UNESCO, 2009). Thus, the values of these parameters in the studied environment were variable, reaching different temperatures in the months studied: (June: 28.3 ° C - 30.1 ° C; July: 28.5 - 30.5; August : 27.6 to 31.6) and the pH (June 6, 73 - 7 62; July : 6.76 to 8.15; August: 6.81 to 8.24). These results demonstrate an environment very rich, whose success and dominance is explained by the low consumption makes the herbivorous zooplankton because of the ability to have this algal group to p toxins roduce (cyanotoxins), which according to the (UNESCO, 2009), They represent a serious problem for other organisms in the aquatic environment and for humans.

It should be noted that low transparency values were also observed in the water column of the Tiscapa lagoon during the three months sampled (approx. 1.6 m), showing a reduced euphotic zone (approx 2.0 m) and a wide area aphotic or dark stratum (approx. 37 m), favoring the flowering of Cyanophyta, which in turn affect their abundance. Importantly large numerical populations *Microcystis aeruginosa* in laguna of Tiscapa in strata from 0 to 39 m depth for different sampled. The contributions populations of this taxon ranged from 56 to 99 % of abundance overall (Table 10), inhibiting the ability photosynthetic other algae, because l shadow or produced in the surface layer of the water surface and in consequence a higher decomposition their dense populations, as well as a greater reduction in the availability of oxygen, degrading the aesthetic quality of the water due to the production of unpleasant odor and taste.

Table 10 . Dominant group and contribution of taxa to the total abundance of phytoplankton at the center point of the Tiscapa lagoon .

Month	Depth	Abundance%

Sampling point		(m)	Dominant group and tax contribution	
Center Point	June	0 m	Cyanophyta	*Microcystis aeruginosa* (70%)
		20 m		*Microcystis aeruginosa* (56%)
		39 m		*Merismopedia tenuissima* (69%)
	July	0 m		*Microcystis aeruginosa* (99%)
		20 m		*Merismopedia tenuissima* (80%)
		39 m		*Merismopedia tenuissima* (57%) *Planktolyngbya* sp (40%)
	August	0 m		*Microcystis aeruginosa* (92%)
		20 m		*Microcystis aeruginosa* (80%)
		39 m		*Planktolyngbya* sp (44%) *Merismopedia tenuissima* (43%)

6.3.1.3 Incidence of PoC or in the Phytoplankton Community

During the second phase of PoCo application, the total abundance of phytoplankton for the months of June, July and August 2020 was highly fluctuating (Table 1 1). It is noticeable, a higher numerical contribution of phytoplankton in July, during the development of the phase without treatment (PoCo) and without Aerator in the Lagoon (Table 10). However, during the three - month study (June, July and August), always predominated Cyanophyta , excelling algal populations of *Microcystis aeruginosa* , species characteristic of a mbientes eutrophicated s .

Table 11 . Total abundance of phytoplankton in the different samples for the center point of the Tiscapa lagoon , 2020 .

Sampling point	Month	Numerical abundance	PoCo application
C entered	June	20 408 241 Ind l^{-1} 70% *Microcystis aeruginosa*	With PoCo and Aerator
	July	122 784 494 Ind l^{-1} 99% *Microcystis aeruginosa*	Without Little and without Aerator
	August	72 559 923 Ind l^{-1} 92% *Microcystis aeruginosa*	With PoCo and Aerator

Importantly different contributions to the abundance Total phytoplankton in the period 2018-2019, which dominated the Cyanophyta for p daub C entered Lagoon Tiscapa , just as observed for 2020 in the June, July and August. The records obtained in 2018-19 were greater in the illuminated surface (0 m), during the month of October (n = 595 485 695 Ind l^{-1}) than in August at 20 m depth (n = 2 291 149 Ind l^{-1}). It is worth mentioning that during this study period (August and October) there was no type of treatment (PoCo) in the Tiscapa lagoon , showing that these communities do not easily present functional changes. Based on this, (Roldan & Ramirez, 2008) mention that the functional biological or morphological forms that are characteristic of aquatic environments do not change easily and are mainly related to the turbulence of the environment and the availability of nutrients.

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It is also significant to mention that during the study period: June, July and August 2020, there is a lower contribution to the richness of green algae (Chlorophyta), compared to the 2018-19 period, where there was no supply of PoCo. In this last period, the species richness that made up the Clorophyta community was 40 taxa (65% of the total richness) showing a significant decrease in 2020 where only 14 taxa were observed, representing 48% of the total taxonomic record.

6.3.2 Zooplanktonic Structure

Among the components that make up the biotic community in an aquatic ecosystem, zooplankton is a fundamental member of bodies of water, ranking among the primary producers and secondary consumers such as fish fry in the trophic chain. Its main ecological importance is the transfer of energy within aquatic systems, its abundance and distribution vary temporarily in response to changes in biotic and abiotic factors, so its composition and abundance change in response to changes in diversity and Phytoplankton abundance (Armengol, 1982; Folt & Burns, 1999; We tzel , 2001). It is for this reason that the study of zooplankton provides invaluable information about the trophic state, the processes of general productivity of a body of water, in addition to providing some members of the zooplankton as biological indicators due to their short life cycle, their sensitivity environmental changes and pollution (Gómez et al. 2013; García, 2015; Wetzel , 2001 ; Roldán and Ramírez, 2008).

6.3.2.1 Community Structure and Species Wealth

The structure of a community has been defined as the way in which organisms are distributed in space to take advantage of the conditions offered by the biotic and abiotic environment. As a consequence, relationships are established regarding the relative contribution of the species. The end result is a community structured in space and time that assumes a defined pattern or series (Begon & Harper, 2000; Smith & Smith, 2007). The general pattern in this aquatic ecosystem consists of a few common and very common species , followed by a few low-abundance and equally common species and a high abundance of rare and occasional species. This structural pattern comuni ty zooplankton of the lagoon Tiscapa evidence some changes compared to those reported historically , all taxa reported are recognized typical mind in tropical waters and eutrophic environments (Appendix, Table 2).

During rainy period June to August a total of 20 registered taxa of zooplankton on two Phylum Rotifera: (n = 14) and Arthropoda (n = 6). In order of importance, rotifers were responsible for shaping the community structure of zooplankton contributing in (12.2 ± 73.5%) of the species, with low variability between periods sampled, meanwhile artrópod or s exhibited a low contribution , being represented by two groups of crustaceans: cladocerans (n = 4) and copepods (n = 2), these contributed from 13 to 35.7% of the total wealth during the study period . These groups are essential for the transfer of energy in the food web. As these are almost exclusively consumers of algae and detritus , they are capable of modeling the development of phytoplankton in natural systems, at the same time, they are favorite prey of vertebrate and invertebrate predators (Dumont , 1994) all the taxa that make up this group are considered common in tropical areas (Annex , Table 2).

In the evaluation of the contribution of the main zooplankton groups to the total richness of species, significant differences (P <0.001) are evidenced (Fig. 16 a), tied to this unidirectional test a Dunn's multiple comparison test was performed that allowed It identified which population groups were dissimilar in relation to their contributions to the total richness of species, showing that there is a difference between rotifers and copepods; rotifers and cladocerans, but not between cladocerans and copepods. On the other hand, and l pattern distribution and behavior of species richness exhibited slight variations spatiotemporal is , (Figure 16). L nonparametric tests as Kruskal Wallis statistics, showed no significant differences between the depths (P = 0.98) (Fig. 16b) and sampled periods with and without treatment (P = 0.711) (Fig. 16c) .

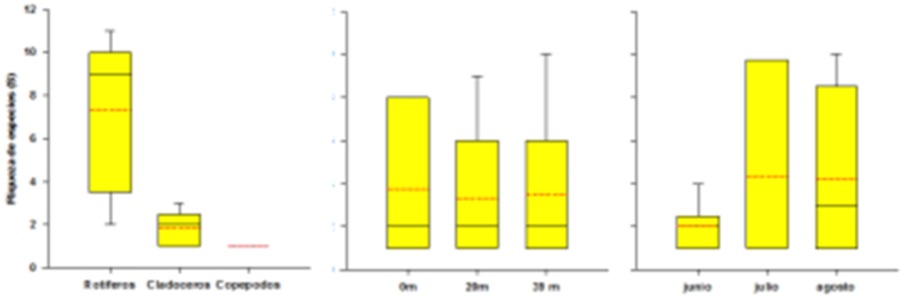

Figure 16 . Behavior of the species richness of zooplankton between groups (a), depths (b) and sampled periods (c), during the rainy season, at the center point of the Tiscapa 2020 lagoon .

Indeed, we can infer that the outstanding taxonomic representativeness of the rotaries is mainly due to their high ecological plasticity , which allows them to adapt to sudden changes in their environment by having a short life cycle with a higher reproduction rate compared to the group of the cladocerans and copepods that are more sensitive, which is why the predominance in the community structure of rotifers is common in tropical and sub-tropical eutrophic waters (Iannacone and Alvariño 2006, Aranguren-Riaño and Monroy-González 2014).

As rotifers are the most diverse group in the zooplankton community, it manages to group the largest number of families (n = 8) and species (n = 14), considering a common and generalized pattern associated with eutrophic bodies of water where contributions to wealth predominate. of many families , especially Brachionidae . This family rallied during this phase of the study (June, July and August), 35% of species being *Brachionus* the most prominent genre aport ando 20% of the total wealth. L os representatives of est and taxon have a high distribution in aquatic environments, therefore, are considered cosmopolitan and planktonic condition that favors the probability of finding them at different depths and areas in aquatic environments (from the coastal strip to open water).

It is noteworthy that the representatives of the genus *Brachionus* , are considered organisms opportunistic r type, because they are able to exploit effectively the conditions of high material availability detrital which are produced by decomposition processes, developed by the community bacteria na associated mineralization of the allochthonous and autochthonous particles suspended in the water column. Therefore, in this aquatic environment in particular are c onsiderados quality indicators highly eutrophic waters . This specific condition is consistent with those reported by other researchers (Ávila, 1973, Pennak , 1978; Ramírez, 1987; Estrada 1995; Ramírez and Díaz, 1996-1997); cited by Roldán & Ramírez, (2008) where they relate these same groups to highly eutrophic environments .

Finally, a retrospective comparison of the total species richness of the zooplankton community , relative to the baseline study of the 2018-2019 period, in contrast to the records of June, July and August 2020 , shows a reduction in the total zooplankton richness. In 29%, rotifers being the group that reflected the greatest transformations in its population , their wealth had a decrease of 44% (n = 14) (Annex, Table 2) . At the same time, it is important to highlight that there was an increase in the number of taxa sensitive to contamination and eutrophication, such as cladocerans, a characteristic group of little altered environments due to their low tolerance to eutrophic conditions. The increase of this group in the number of zooplanktonic taxa was 85% (n = 4), compared to the 2018-19 reports (n = 1).

These changes in community structure of zooplankton suggest a brief and subtle restoration of health and trophic status of the ecosystem, based on the basis of the increase of sensitive taxa to contamination associated with eutrophication of l ago, in addition to the substantial reduction of the tolerant taxa of eutrophic condition primarily from the group of rotifers. However, we show that there is still a dominance pattern of rotifers in the community structure, a characteristic condition of eutrophic waters, which means that the processes of restoration and change of the trophic state are slow and gradual.

6.3.2.2 Numerical abundance of zooplankton .

In the contribution between groups , according to the Tukey test, there were significant differences (P = 0.003), that is, between: rotifers and cladocerans, but not, between rotifers and copepods, nor between copepods and cladocerans (Fig. 17a) . The behavior of the abundance and distribution of zooplankton no evidence rum significant differences spatiotemporal. The non-parametric Kruskal Wallis tests prove this condition spatially (depth; P = 0.896) (Fig. 17 b) and temporally (periods; P = 0.425) (Fig. 17 c) .

Regarding the contribution of the main heterotrophic groups, a prevailing and maintained pattern of zooplankton with a smaller spectrum is observed, such as: rotifers and this larval god of copepods (Fig. 17b). It should be mentioned that the numerical predominance of rotifers in average proportions of (50.6 ± 33.4)% (Fig . 17 c) in association with the larval and juvenile stages of copepod (36.6 ± 34.9)% is common in tropical freshwater environments, be these lakes, lagoons, reservoirs, rivers or streams (Moreno, 2003) (Fig. 17d . The high density of zooplankton with a smaller spectrum that includes rotifers and immature forms of copepods, (91.9 ± 10.8)% are the result of continuous reproduction, this being of great importance for the community structure of zooplankton, in relation to population dynamics and trophic aspects.

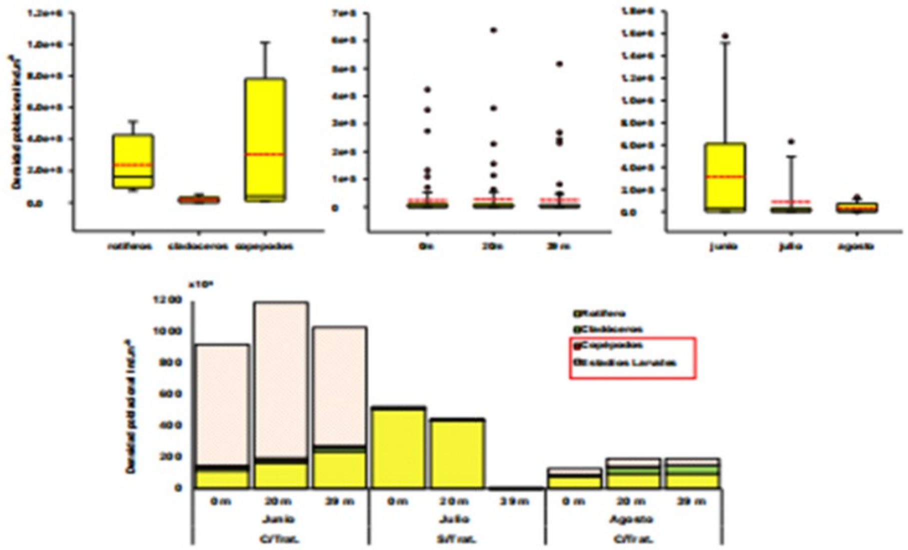

Figure 17 . Behavior of the total abundance of zooplankton and the contribution of its main groups during the sampling campaign for the months of June, July and August 2020, at the center point in the Tiscapa lagoon .

In the particular case of rotifers, their abundant populations reached an average of 50.6 ± 33.4% are associated with their ability to be opportunistic and strategic organisms . Furthermore, its small size, short life cycle, wide tolerance to a variety of environmental factors, and efficient filtration mechanisms play a very significant role in this abundance pattern. (Neves, Roche, & Pinto, 2003) . In this sense, Edmondson , 1946 and 1965; King, 1967; Halbacch and Halbach and Keup , 1974; cited by Wetzel (2001) , they agreed that the rate of reproduction of rotifers is closely related to the amount and abundance of food available. At the same time, it is important to mention that the relative proportion of non-consumable phytoplankton increases with eutrophy , suggesting that the more eutrophic lakes generate higher concentrations of debris that would favor more the presence of rotifers (Pace, 1986 ; Payne, 1986) .

Regarding the copepods, several factors could be promoting the high abundances of larval and juvenile stages of this group. That is, they are ecologically considered as K strategist, which successfully allows their prevalence in aquatic environments, since it guarantees population control depending on biotic conditions (availability of resources, competition, predation) and fluctuating abiotic conditions (the which eutrophied water bodies are subjected). This adaptive advantage allows this population to compete for edible food resources with other representatives of the same community. Furthermore, in the presence of adverse abiotic conditions, they are capable of stopping their development, promoting a diapause state that guarantees their prevalence for long periods of time, restoring their growth when conditions allow it.

In general , the dominance pattern of certain groups of zooplankton in the Tiscapa lagoon obeys more a condition of adaptation and advantages in the use of available resources, resulting in unequal increases in zooplankton populations , where the main beneficiaries they are the smaller zooplankton (rotifers and larval stages of copepods).

6.3.2.3 Diversity of species .

To determine the diversity of the species within the system, the Shannon-Weiner index was used (Brower & Zar, 1977; Franco et al., 1985; Moreno, 2001), which reflects the heterogeneity of a community on the basis of two factors: the number of species present and the relative abundance. This index was used as it assumes that individuals are randomly selected and that all species are represented in the sample .

Comparing with the results obtained in the study period 2018-19, it is shown that the diversity of the zooplankton of the lagoon has not undergone changes over time, persisting a stress behavior that is reflected in the low values of diversity. (1.2 ± 0.4 bits.ind), the values ranging from 0.77 bits.ind to 1. 74 bits.ind maximum (Fig. 18 a). According to Dole-Oliver, Galassi, Marmonier, & Des Chatelier (2000), they consider low values of diversity when environmental conditions are altered, being an indication of an unstable community controlled by environmental factors, product of alterations of the physical and chemical environment of the environment, in this sense Wetzel (2001), indicates that the low diversity of species in freshwater bodies is strongly linked to their eutrophic state.

Statistically, it was verified through the analysis of variance that there are n significant differences in diversity between the sampled periods ($P = <0.001$); $\alpha = 0.050$: 1.000. (Fig. 18a). Complementarily, a multiple comparison was evaluated using the Holm- Sidak statistical test , to find which periods were dissimilar, showing that all periods reflect significant differences. However, the diversity between depths does not show significant changes ($P = 0.709$) (Figure 18 b) . This behavior indicates that the ecosystem continues to be stressed by anthropogenic activities , variable environmental conditions and by the mixing pattern of the lagoon as a physical factor . This is evident when comparing the maximum expected diversity against the calculated one (H '), on average it oscillates in (2.2 ± 0.3) bits.ind , and at most it registers 2.6 bits.ind , this last value would be the ideal value for this aquatic environment (Fig. 18b).

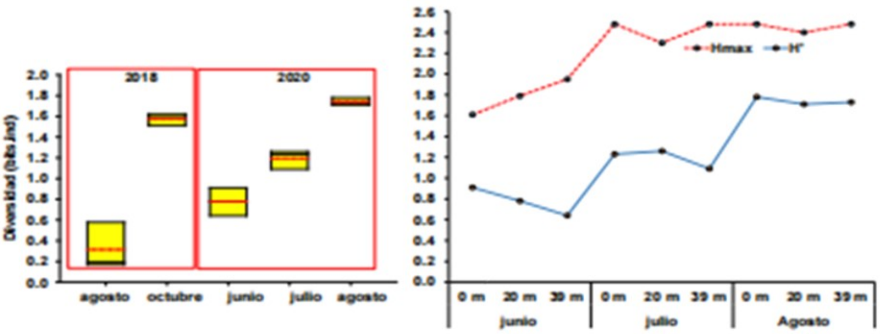

Figure 18 . Behavior of the diversity of species (H ') temporal space of the zooplankton community of the Tiscapa lagoon 2020.

6.3.2.4 Evaluation of the biological activator of PoCo in the Zooplankton Community

Species richness and abundance of the community zooplankton studied during June, July and August 2020 show no significant differences in space and time during the treatment phase (POCO) and without it (Figures 16 and 17). However, there are significant differences between the main groups of zooplankton, maintaining a characteristic distribution and permanence pattern of eutrophied waters , where the abundance of smaller zooplankton prevails, including rotifers as eutrophy-tolerant species.

It is important to highlight that during the period without treatment (July), changes in the abundance and dominance of rotifers were observed, which represented 97.6% of the total abundance. This condition propici or an increase in intraspecific powers s by the use food between zooplankton smaller (rotifers) with the larger species (Cladocera and copepods), particularly the rotary took advantage because they are more efficient filtering their food, this condition caused a decrease in the abundance of larger zooplankton.

Later, once the experiment was resumed for a period of 30 days, an increase of 22% in the abundance of the largest species was observed, mainly cladocerans, this group is characterized by being sensitive to eutrophic conditions, this unexpected increase Its abundance could be associated with the fact that in the aquatic environment there is a momentary decrease in filamentous algae, this situation is complemented by a decrease in the abundance of rotifers and larval and juvenile stages of copepods, which together allowed the cladocerans to will develop.

In general, during the periods with treatment versus without treatment, we observe that the changes are not yet substantial. The Shanonn diversity index shows that the aquatic system is still subject to the condition of hypertrophy, (Fig. 18), so the structural pattern is still characteristic of hypertrophic environments. It is worth mentioning that the changes in the communities during the restoration processes are slow and gradual.

6.4 **Physicochemical Characterization of Sediments**

6.4.1 Moisture percentage

The sediments sampled at the site located 800 m from the Aerator Point showed a moisture content of 79.74%. The results show that the sediments studied have a high moisture content in the first 10 cm of depth.

6.4.2 Granulometry

In the granulometric composition of the sediments, it was silt, clay and sand with 52, 38 and 10%, respectively (Table 12). The sand content was found with a proportion of 10% made up of coarse materials. The behavior of silt and clays showed the influence of rainfall during the rainy season that increased the transport of sediments and the domestic and industrial wastewater that drains from the channels to the Tiscapa Lagoon. On the other hand, the distribution of sand in the Punto Aireador site is possibly due to materials from the Tiscapa channel that are transported from the upper and middle part of the micro-basin from soil erosion due to precipitation during winter.

According to the results of the granulometry, the distribution of the grain size in the study area presented a silty clay loam texture (Table 12). The behavior of the granulometry in the sediments of the Aerator Point site is possibly due to materials originating from erosion that arrive by surface runoff from the upper part of the micro-basin through the channels that drain into the water body, to the wastewater domestic and industrial, associated with changes in the flow regime that increases considerably due to heavy rains, transporting more dissolved material in suspension to the lagoon.

Table 12. Results of granulometry and organic matter in the sediments of the Tiscapa lagoon.

Sampling date	Sampling sites	Moisture Content (%)	Granulometry Sand (%)	Slime (%)	Clay (%)	Texture	Organic material (%)	Total phosphorus (mg g^{-1})
June 2020-06-24	Aerator Point	79.74	10	52	38	Silt Clay Loam	5.70	0.065

6.4.3 Organic matter content

The content of organic matter found in the sediments was 5.70%, this percentage of organic matter is probably due to the deposit of fine materials such as silt and clay from soil losses in the upper part of the micro-basin and from wastewater domestic and industrial that drain into the Tiscapa channel (Table 12). Comparison of the content of total organic matter with the granulometry in this study indicates that there is a relationship between these variables. Therefore, the amount of organic matter conserved in the sediments depends, among others, on the texture of the sediments and the relationship between the rates of input and decomposition of organic matter (Pineda, 2009).

6.4.4 Total phosphorus

The total phosphorus concentration (FT) was 0.065 $mg.g^{-1}$ in the sediments collected at the site located at the aerator point. The low value of the FT is probably due to two causes; the first one is the migration towards the deeper layers of the sediment in the form of slightly soluble hydroxide and the other possible cause could be that due to the anoxic conditions of the water column in contact with the sediment, the FT has passed to this in the form of soluble phosphates . According to Hart et al. (1976) , under certain particular conditions of anoxia and change in redox potential, phosphorus can be released from sediments to the water column, increasing concentrations, being used as a nutrient for phytoplankton growth. Although it could really be due to a combination of the two processes; sedimentation towards the deeper layers and release towards the water column. According to Roldan & Ramírez (2008), the mechanism that causes phosphorus to precipitate and return it back to the water depends on a series of physical, chemical and biological factors. Among the elements that intervene in the precipitation of phosphates or their immobilization we find iron, aluminum, sulfates, organic compounds, pH and oxide reduction conditions.

7. Preliminary conclusions

1. The waters of the Tiscapa lagoon are classified as waters of medium mineralization and medium alkalinity (surface and 20 m) and high alkalinity at the bottom in August . The oxygen distribution was heterogeneous, finding a poorly oxygenated water column. The decrease in pH from the surface to the bottom indicates a great influence of microbial respiration processes, whereas the increases in pH at the surface were influenced by photosynthetic activity.

2. After the additions of the PoCo product since June, both BOD and COD have decreased concentrations when comparing the values of this sampling with that of August 2018, observing an evident improvement in water quality .

3. The BOD ,/COD ratio in the waters of the lagoon for the months under study indicates the dominance of organic matter in the biodegradable range in 77.7% and 22.2% was characterized by being very biodegradable.

4. Ammonium was found in very low concentrations on the surface and in the middle layer, at the bottom it presented the highest concentrations, possibly as a result of the reductive environment generated by the decomposition of organic matter. The distribution pattern of total phosphorus was heterogeneous, with relatively low concentrations observed at the surface and at medium depth, and very high concentrations at the bottom of the lake, attributable to the release of the phosphate ion from the sediments facilitated by the low concentration of oxygen or its recycling. by microbial action. The Laguna de Tiscapa with the

Salas & Martino model based on total phosphorus resulted in a mostly hypereutrophic .

5. The microbiological analyzes evidenced the presence of contamination indicator organisms, which is influenced by the dragging of diverse material, be it plant and animal, thus affecting the quality of water for its potential uses (recreation and agriculture). On the other hand, a decrease in thermotolerant coliforms for the month of August was observed , this may be due, on the one hand, to the little drag of alien material that reaches the lagoon through the channel or it could be the effect that PoCo has , aeration or by increasing the volume of the cuvette in thermotolerant coliforms .

6. With the application of different concentrations of the PoCO product for the months of June, July and August, the phytoplankton community in Laguna de Tiscapa maintained a dominance (70% - 99%) of the group of Cyanophytas (*Merismopedia tenuissima* , *Microcystis aeruginosa* , *Oscillat oria limosa* and *Planktolyngbya* sp) , a characteristic group of eutrophic environments and waters degraded by the effects of pollution of anthropogenic origin.

7. The dominance pattern of zooplankton in the Tiscapa lagoon is due more to an adaptation condition and advantages in the use of available resources, resulting in unequal increases in zooplankton populations , where the main beneficiaries are the smaller zooplankton species. (rotifers and larval stages of copepods).

8. The zooplankton community of Tiscapa, with the application of different concentrations of the PoCo product, shows subtle changes, but when the treatment is stopped, increases in the abundance of rotifers are experienced. The c retrospective OMPARISON community zooplankton in the period 2018 to the current study which is being used biological activator, shows a clear reduction of the indicator species of eutrophication and a slight increase of susceptible species and indicator of improvement of the system.

9. Conclusively, the processes of restoration and improvement of the trophic state of the Tiscapa lagoon have been taking place gradually, the diversity indicators and the structural patterns of the heterotrophic community are still characteristic of enriched environments of degraded waters due to the effects of source pollution. anthropogenic.

10. The granulometry and organic matter in the sediments is due to materials coming from the upper part of the basin, from the channels that drain into the body of water, from the domestic and industrial wastewater of the Tiscapa channel , construction works in the city associated with changes in the flow regime that increases considerably due to heavy rains, transporting more

dissolved material in suspension to the lagoon. In sediments, the silt fraction predominated, which is related to the content of organic matter.

11. The phosphorus concentration may be due to the sedimentation into deeper and release layers to the colum na water.

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9. Annexes

Table 1. Taxonomic list of the phytoplankton present in the Tiscapa lagoon during the months of June, July and August 2020 with and without application of the PoCo product.

Cyanophyta	**June ***	**July ****	**August ***
Merismopedia tenuissima	x	x	x
Microcystis aeruginosa	x	x	x
Silty oscillatoria	x	x	x
Planktolyngbya sp	x	x	x
Chlorophyta			
Actinastrum hantzschii	x	x	
Ankyra sp	x	x	
Closterium limneticum	x		
Closterium parvulum		x	x
Coelastrum astroideum	x	x	x
Coelastrum indicum	x	x	x
Dictyosphaerium pulchellum	x	x	
Micractinium pusillum	x	x	x
Monoraphidium contortum	x	x	x
Oocystis lacustris	x	x	x
Pandorina sp	x	x	x
Scenedesmus armatus	x	x	x
Scenedesmus semipulcher	x	x	x
Scenedesmus sp		x	
Bacillariophyta			
Achnanthes sp		x	x
Aulacoseira granulata	x	x	x
Cyclotella meneghiniana	x	x	x
Cymbella sp	x		x
Fragilaria crotonensis			x
Gomphonema sp			x
Navicula sp			x
Nitzschia sp	x	x	x
Pinnularia sp			x
Synedra ulna	x	x	x
Cryptophyta			
Cryptomonas sp	x	x	x

***: c / trat**
****: s / trat**

Table 2 . Zooplankton community structure reported for the sampling campaigns during the rainy period from June to August 2020 in the Tiscapa lagoon . (+) Presence; (-) Absence.

PHYLUM	CLASE	SUB-CLASE	ORDEN	FAMILIA	ESPECIES Y/O GENERO	2018-2019	2020	2020		
								Junio**	Julio*	Agosto**
Rotifera	Eurotatoria	Digononta	Bdelloidea	Bdelloidea f.n.i	Bdelloidea g.n.i	+	+	+	+	+
		Monogononta	Ploima	Brachionidae	*Anuraeopsis sp*	-	+		+	+
					Brachiounus angularis	+	+		+	
					Brachiounus sp	+	-			
					Brachiounus caudatus	+	+		+	+
					Brachiounus calyciflorus	+	+	+	+	+
					Brachiounus havanaensis	+	-			
					Brachiounus leydigi	+	+		+	+
					Keratella americana	+	-			
					keratella cochlearis	+	-			
					keratella tropica	+	-			
					Keratella quadrata	+	-			
					Epiphanes macrorus	-	+		+	
					Euchlanis sp	-	+		+	
				Lepadellidae	*Colurella sp*	-	+		+	
				Synchaetidae	*Polyarthra sp*	-	+	+	+	+
				Lecanidae	*Monostyla lunaris*	+	-			
					Monostyla bulla	+	-			
					Monostyla sp	+	-			
					Lecane monostyla	+	-			

		Lecane sp	+	+	+	+	+
	Trichocercidae	*Trichocerca sp*	+	-			
	Asplanchnidae	*Asplanchna sieboldi*	+	-			
Flosculariaceae	Hexarthridae	*Hexarthra intermedia*	+	-			
		Hexartra sp	+	+	+	+	+
	Trochosphaeridae	*Filinia longiseta*	+	-			
		Filinia opoliensis	+	-			
	Flosculariidae	*Ptygura sp*	+	-			
	Conochilidae	*Conochilus sp*	+	+		+	+
	Testudinellidae	*Testudinella patina*	-	+		+	+
Collothecacea	Collothecidae	*Collotheca sp*	+	-			

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PHYLUM	CLASS	SUB-CLASS	ORDER	FAMILY	SPECIES AND / OR GENDER	2018 -2019	2020	2020 June **	2020 July*	2020 August**
Arthropoda	Crustacea	Branchiopoda	Cladocera	Moinidae	*Moina micrura*	•	•	•		
				Daphniidae	*Ceriodaphnia cornuta*	-	•	•	•	•
				Sididae	*Diaphanosoma brachyurum*	-	•			•
				Chydoridae	*Alona sp*	-	•			•
		Maxillipoda	Cyclopoid	Cyclopidae	*Mesocyclops edax*	•	•	•	•	
					Mesocyclops thermocyclopoida	•	-			
					Mesocyclops aspercornis	-	•			•
Total wealth						28	twenty	8	16	fifteen
Rotifers total wealth						25	14	5	14	eleven
Cladocera total wealth						1	3	2	1	3
Total wealth Copepods						2	2	1	1	1

Treatment (PoCo), applied by the Mayor's Office of Managua during the sampling periods of : June, August

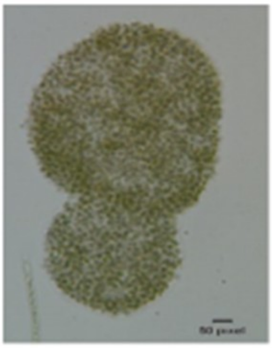
* without treatment (15 days), July
** with treatment and aeration (30 days ~)

UNIVERSIDAD NACIONAL AUTÓNOMA DE NICARAGUA, MANAGUA
UNAN - MANAGUA

PODER CIUDADANO
ALCALDÍA DE MANAGUA
BUEN GOBIERNO!

CENTRO PARA LA INVESTIGACIÓN EN RECURSOS ACUÁTICOS DE NICARAGUA
CIRA/UNAN-MANAGUA

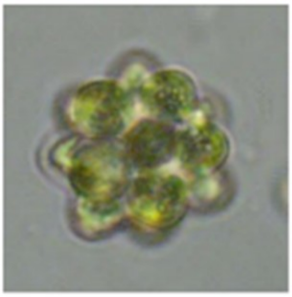
Photographs 1 : Cyanophyta

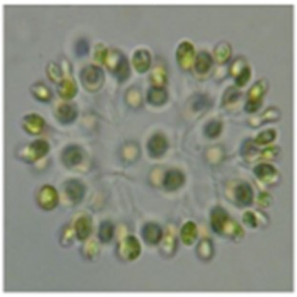

Microcystis aeruginosa


Merismopedia tenuissima

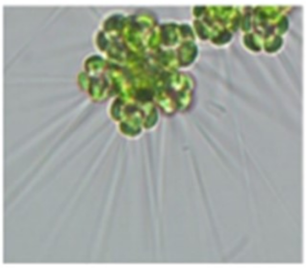

Oscillatoria limosa

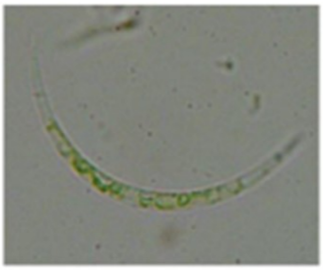
Photographs2 : Chlorophyta


Coelastrum sp


Dictyosphaerium sp


Actinastrum sp

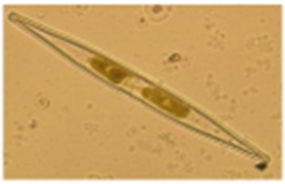

Micractinium sp


Closterium sp

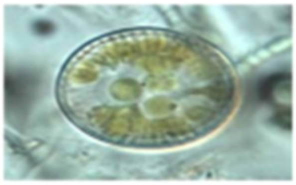

Scenedesmus sp

Photographs : Bacillariophyta

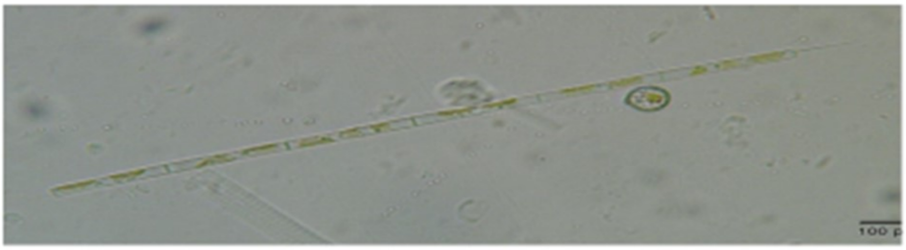
Nitzschia sp



Synedra sp



Cyclotella sp



Aulacoseira sp

10. Glossary

Anaerobiosis: The ability of some organisms, such as fungi, bacteria, parasites, etc., to live without free molecular oxygen. Anaerobiosis is synonymous with life in the absence of free oxygen.

Anoxic: Anoxic waters are areas of seawater, fresh water, or groundwater in which dissolved oxygen is depleted. This condition is generally found in areas with limited water exchange and with eutrophication processes in progress.

Clinógrade curve : vertical oxygen distribution curve where the content is relatively higher near the surface, coinciding with the stratum where photosynthetic activity takes place, decreasing with depth. This distribution pattern is observed in stratified and eutrophied water bodies.

Epilimnio: warm surface layer in a thermally stratified lake, of variable depth depending on the spatial variation of temperature.

Trophic state: phase in which an aquatic ecosystem is found with respect to the concentration of nutrients and the level of primary productivity.

Eutrophic: advanced phase of the eutrophication process of an aquatic ecosystem, characterized by the high concentration of nutrients and phytoplankton biomass.

Eutrophication: it is the natural and / or artificial process of enrichment of aquatic ecosystems by phosphorus and nitrogen from the drainage basin that gradually leads to an increase in biological production, a decrease in diversity and a loss of water quality.

Phytoplankton: photosynthesizing autotrophic plankton with chlorophyll *a* , formed by various groups of microalgae (eukaryotes) and cyanobacteria (prokaryotes). Some microalgae can be mixotrophic. They inhabit all continental and marine water systems. They cover a wide range of sizes from almost 1 µm to visible to the naked eye (almost 3 mm).

Flowering: accelerated short-term growth of one or more populations of cyanobacteria or eukaryotic algae. Typically in phytoplankton. In general, the blooms are made up of one or a few species and can last from a few days to the whole year.

Hypereutrophic: more advanced phase of the eutrophication process of an aquatic ecosystem, characterized by excess nutrients and high productivity, especially of algal biomass, which stimulates respiration, bringing the ecosystem to anaerobic conditions, causing the massive death of organisms.

Hypolimnius: colder and denser layer of water, deep, below the thermocline, in a thermally stratified lake.

Lentic: term used in limnology to refer to inland surface water ecosystems such as lakes, ponds and pools.

Mesotrophic: intermediate trophic state in the eutrophication process of an aquatic ecosystem.

Metalimnio: zone of maximum temperature change of a thermally stratified lake.

Trophic level: position in the trophic web defined by the number of energy transfers to that position.

Oligotrophic: initial phase of the eutrophication process of an aquatic ecosystem, characterized by the low concentration of nutrients and low phytoplankton biomass.

Oxycline: layer of abrupt discontinuity of oxygen, where the concentration falls to the value of 1.0 mg l^{-1} and even lower.

Plankton: communities of organisms that live in suspension in the water column. Three basic communities are identified in limnic systems: bacterioplankton, phytoplankton, and zooplankton. Ichthyoplankton (fish larvae) are also part of plankton.

Thermocline: it is the horizontal plane in the water column where the change in temperature with depth is maximum, enough to cause an abrupt change in its density and make it difficult to mix. In general, the thermocline is recognized when there is a thermal discontinuity greater than 1 ° C / meter.

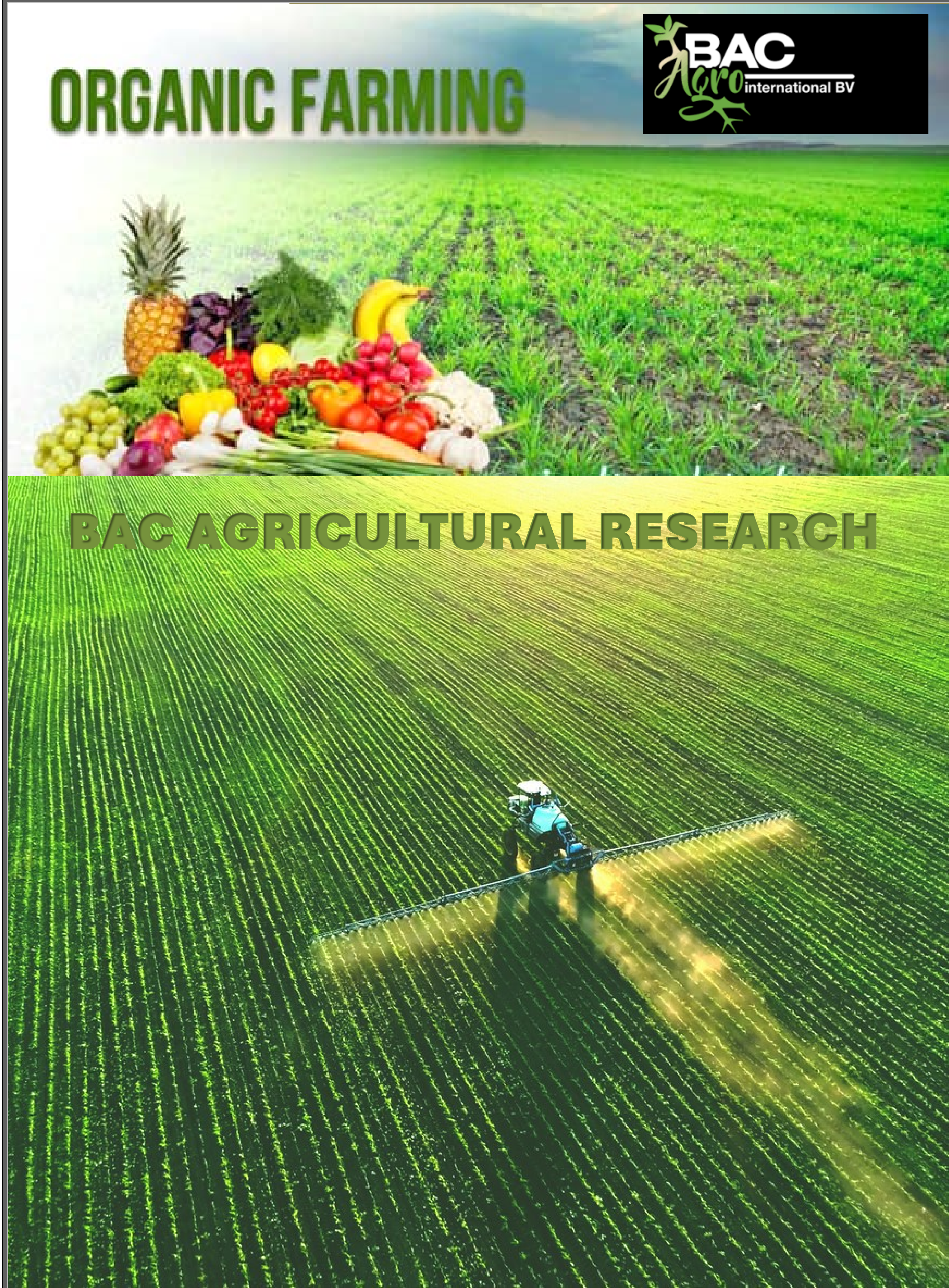
Aphotic zone: zone in which the development of photosynthetic processes is not possible, since less than 1% of sunlight penetrates them.

Euphotic zone: Surface layer of a body of water where enough light penetrates to allow photosynthesis to occur, to a depth where the intensity of the light decreases to 1% of incident light.

Limnetic zone: corresponds to the zone of open waters that extend to the depth where the level of compensation is reached, that is, where photosynthesis balances respiration. Below this level, and due to the scarcity of solar radiation, it is of difficult productivity.

Littoral zone: transitional area between terrestrial and aquatic systems (ecotone) and is considered as an ecological frontier characterized by intense processes of matter and energy exchange.

Zooplankton: plankton that includes heterotrophic protozoa (ciliates and flagellates) and metazoan animals (rotifers and microcrustaceans). They are the main predators of phytoplankton and inhabit all inland and marine water systems. They have variable sizes: from a few microns long to 3 mm.


ORGANIC FARMING
BAC Agro international BV
BAC AGRICULTURAL RESEARCH

GERMINATION OF SEEDS IN LABORATORY CONDITIONS

Research Collaboration with the University of Agronomic Sciences and Veterinary Medicine of Bucharest

We are pleased to announce a joint research collaboration between **Bac Agro International** and the **University of Agronomic Sciences and Veterinary Medicine of Bucharest**, focusing on the effectiveness of our products: **Bio Seed** and **Bio Roots** stimulators.

The University conducted the study on the following crops:

1. Tomato
2. Eggplant
3. Bell Pepper
4. Green Salad
5. Artichoke

The results demonstrated **significant improvement** in plant development and overall health when treated with Bac Agro International's **Bio Seed** and **Bio Roots** products, compared to both the untreated control groups and those treated using conventional chemical methods.

This collaboration confirms the effectiveness of our organic solutions in promoting sustainable and healthy crop growth.

Registered by
The Vegan Society

Tomato Seed Research

Two treatment method variants were considered, concerning the effect of Xseed product on the germination of tomato seeds. These were:
I.- testing germination on paper substrate, with two variants - chemically treated seeds and untreated;
II.- testing germination of tomato seeds sown in peat substrate, also with two variants - chemically treated and untreated seeds.

TREATMENT METHOD VARIANTS
I. GERMINATION OF TOMATO SEEDS ON A FILTER PAPER SUBSTRATE
a. Submerging for 60 minutes, before sowing of tomato seeds, chemically untreated, with Xseed product in various concentrations
V1 - Control - submerging the seeds in distilled water
V2 - submerging in Xseed solution of concentration C1;
V3 - submerging in Xseed solution of concentration C2;
V4 - submerging in Xseed solution of concentration C3;
Soaking of seeds was begun on the 13th of July 2010

b. Submerging for 60 minutes, before sowing of chemically treated tomato seeds, with Xseed in various concentrations
V1 - Control - submerging the seeds using distilled water;
V2 - submerging in Xseed solution of concentration C1;
V3 - submerging in Xseed solution of concentration C2;
V4 - submerging in Xseed solution of concentration C3;
Soaking of seeds begun on the 13th of July 2010

II. GERMINATION OF TOMATO SEEDS ON PEAT SUBSTRATE
a. Treatment by humectation of peat substrate after the sowing of tomato seeds (chemically untreated) using various concentrations of Xseed product:
V1 - Control - substrate watered with distilled water;
V2 - substrate watered using Xseed solution of concentration C1;
V3 - substrate watered using Xseed solution of concentration C2;
V4 - substrate watered using Xseed solution of concentration C3;

Chemically treated seeds
b. Humectation of peat substrate after the sowing of tomato seeds (chemically treated) using various concentrations of Xseed product:
V1 - Control - using distilled water;
V2 - substrate watered using Xseed solution of concentration C1;
V3 - substrate watered using Xseed solution of concentration C2;
V4 - substrate watered using Xseed solution of concentration C3;
2
Sowing done on 16.07.2010 .
The germination was tested according to the standard for determining the germination of tomato seeds, in a germinator, at 30oC during the day and 20 oC at night. Germinative energy readings were taken after three days, and the final reading after 15 days.
The following was determined:
- Number of seeds germinated after 3, 6, 8 and 15 days;
- Length of roots;
- Length of stem;
- Rate of growth of roots and stem;
- Statistical interpretation of results using analysis of variance.

RESULTS
Both the chemically treated and the untreated seeds exhibited germination rates of over 15% (V4) 3 days after sowing, when watered with various concentrations of Xseed solutions. All seeds treated with Xseed solutions (seed submerging and wetting of substrate) exhibited germination rates of 87% after 6 days since sowing. Germination rates were at a maximum on 21.07.2010 (8 days since sowing), for all variants where the Xseed product was used. Germination rates for the control variants were much lower than the other variants (table1).
Number of germinated seeds and germination percentage for tomato seeds in controlled conditions.

Variant - seeds submerged for 60 minutes prior to start of germination period	Number of germinated seeds -pcs-				Total seeds per repetition
	After 3 days since sowing	**6 days since sowing**	**8 days since sowing**	**15 days since sowing**	
	16.07.2010	19.07.10	21.7. 2010	28.07.10	
V_1 Ctrl Gr					
Seeds submerged in distilled water	7	12	78	90	100
Chemically treated seeds submerged in distilled water	0	18	65	92	100
V_2 - C1					
Untreated seeds	21	73	95	95	100
Treated seeds	18	81	94	96	100
V_3- C2					
submerged seeds	17	82	92	92	100
Treated seeds	16	87	91	91	100
V_4 - C3					
submerged seeds	15	87	94	94	100
Treated seeds	16	83	95	95	100

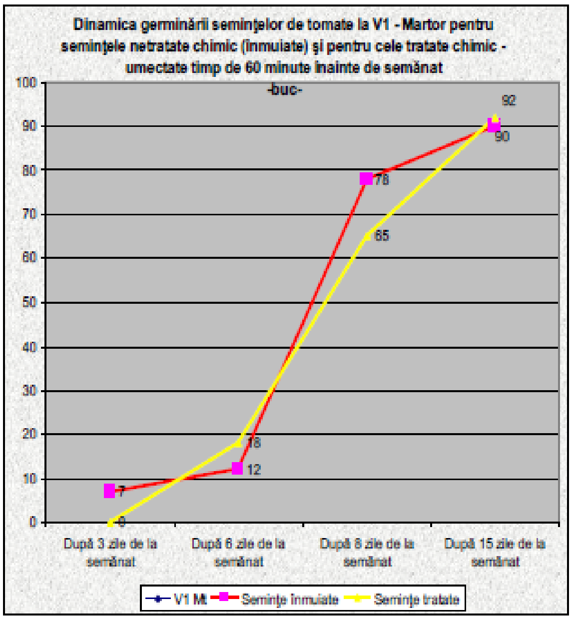


Fig. 1.

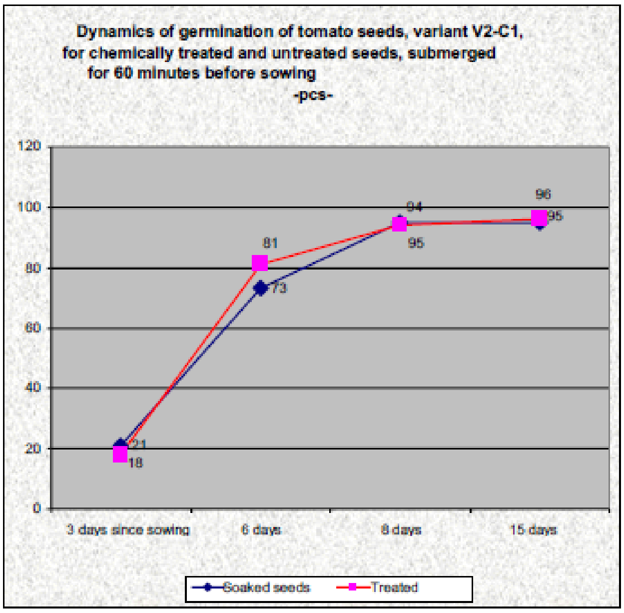


Fig. 2.

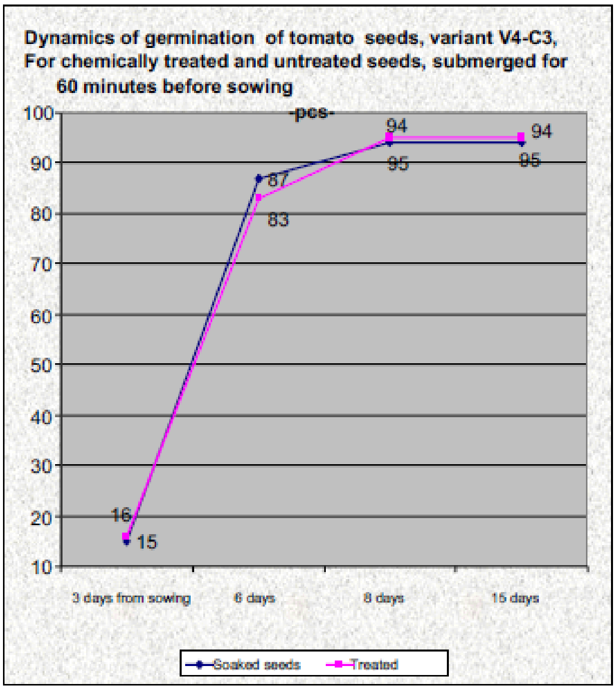


Fig. 3.

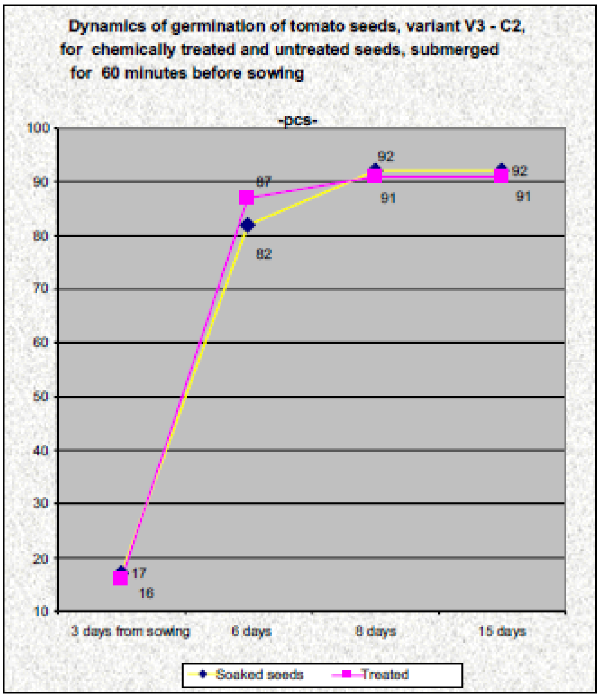


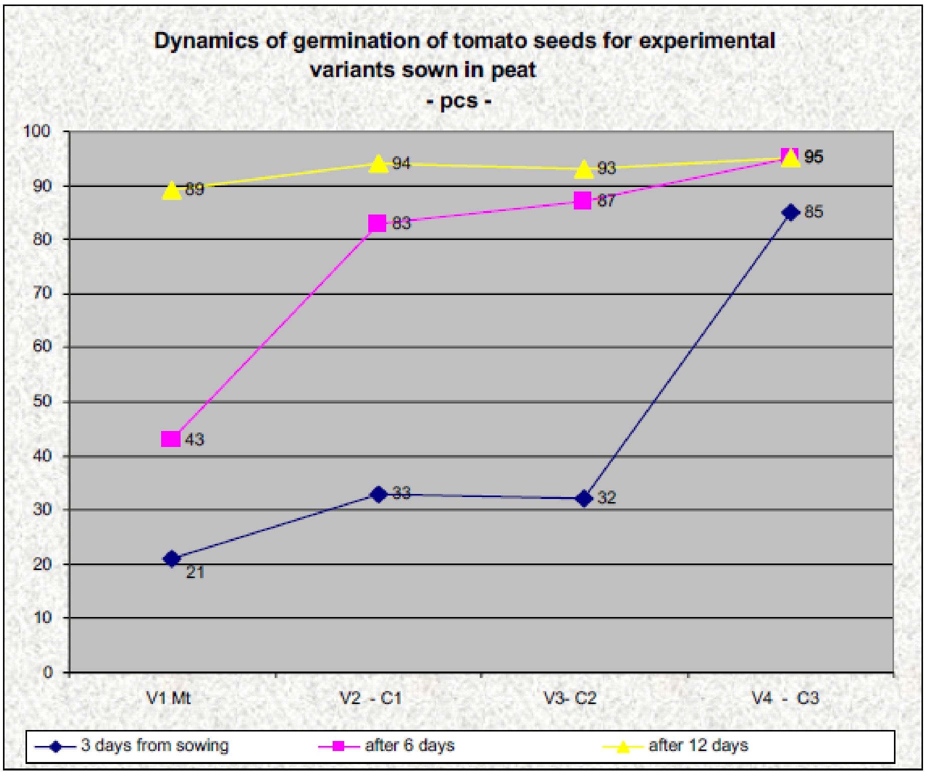
Fig. 4.

In the case of the variant of seeds sown directly into peat substrate, after 3 days it was noted that 85% of V4-C3 untreated seeds germinated, and 95% after 6 days. V1-control germination after 3 days was at 21% and 43% after 6 days (table 2).
The use of Xseed shortened the germination time for tomato seeds by about 6 days, in the case of C3 concentration Xseed.
Xseed did not influence germination rates for seeds that were chemically treated.

Table 2
Number and percentage of germinated tomato seeds - peat substrate

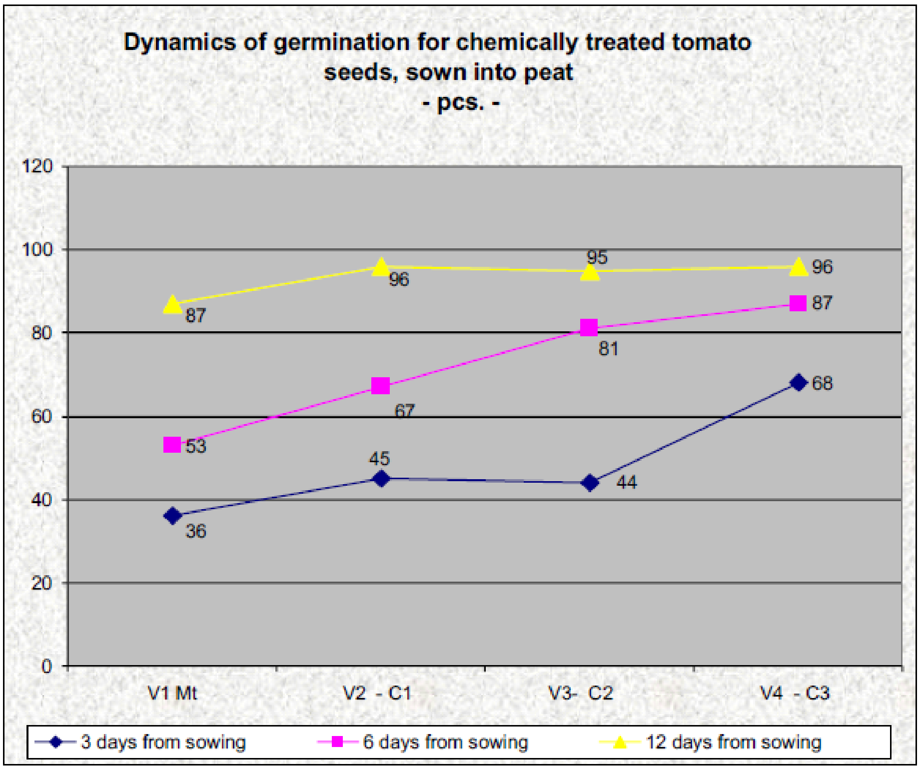
Variant	**Sown**	Number of germinated seeds -pcs-			Total seeds per repetition
		After 3 days since sowing	**After 6 day**	**After 12 days**	
	16.07.2010	19.07.10	21.7. 2010	28.07.10	
V_1 Mt					
Seeds sown in peat	Sown on 16.07.2010	21	43	89	100
Chemically treated seeds, sown in peat		36	53	87	100
V_2 - C1					
Seeds sown in peat	Sown pe 16.07.2010	33	83	94	100
Chemically treated seeds, sown in peat		45	67	96	
V_3- C2					
Seeds sown in peat	Sown pe 16.07.2010	32	87	93	100
Chemically treated seeds, sown in peat		44	81	95	
V_4 - C3					
Untreated seeds sown in peat	Sown pe 16.07.2010	85	95	95	100
Chemically treated seeds, sown in peat		68	87	96	

Fig. 5.



Dynamics of germination of tomato seeds for experimental variants sown in peat
- pcs -
100
90
80
70
60
50
40
30
20
10
0
89
94
93
95
83
87
85
43
33
32
21
V1 Mt
V2 - C1
V3- C2
V4 - C3
3 days from sowing
after 6 days
after 12 days

Fig. 6.



Dynamics of germination for chemically treated tomato seeds, sown into peat
- pcs. -
120
100
80
60
40
20
0
87
96
95
96
87
81
68
67
53
45
44
36
V1 Mt
V2 - C1
V3- C2
V4 - C3
3 days from sowing
6 days from sowing
12 days from sowing

On 19.07.2010 (6 days after sowing), it was noted that the lowest germination rate was exhibited by Variant 1 - the control group, but the treated variants had a higher germination rate, which means that many seeds almost reached their maximum germination point since 19.07.2010 . On 21.07.2010 - 8 days after sowing, the percentage of germinated seeds, compared with the control was between 104.2% for V2 - chemically untreated seeds and 121.5 for V4-C3 - chemically treated seeds (table 3).

Statistically, there are significant differences in the germination of all variants treated with the Xseed product (tables 4 and 5).

The variant in which sowing was done in peat, after three days the percentage of germination was 85% for V4-C3 chemically untreated seeds and 68% for treated seeds. It is considered that the treatment with Xseed did not affect germinative energy of chemically treated tomato seeds (table 6).

6 days on sowing, a maximum of 95% of the V4-C3 chemically untreated seeds had already germinated. This is of course positive, as when seedlings are obtained by sowing and transplanting, the seedlings can be obtained about 6 days earlier. The same is observed for seeds sown in peat cells.

The data in table 6 also shows that Variants 1 - control exhibited the lowest germination rate - 43% for the untreated and 53% for the chemically treated seeds. Compared with the controls, on 21.07.2010, germination rates were very high - 304.8 times for non-chemically treated seeds and 117.6 times for treated seeds (V3-C2).

Germination rates after 12 days (28.07.2010) was a maximum of 89% and 87% for the two controls which leads to delays in obtaining seedlings (table 6). Data in table 7, 8 and 9 shows that on 19.07.2010, as well as 21.07.2010 and 28.07.2010 - after 3, 6 and 12 days respectively, there were statistically significant differences between germination rates of tomato seeds.

On seeds germinated on 16.07.2010, the length of the radicle was between 2 and 3mm for the controls (table 10) and 4-5mm for the treated variants.
It is noted that the radicular system was not affected by treatments with Xseed (tables 11, 12, 13 and 14). Also, untreated seeds' root growth did not exhibit any negative reaction to Xseed treatments. After 15 days, variants treated with Xseed showed radicles longer by up to 172.22% than control variants.

Tomato roots (chemically untreated seeds) exhibited a statistically significant growth rate compared to V1 - control, when seeds were soaked for 60 minutes. The growth rate difference was not statistically significant for the chemically treated seeds (table 14).
The variant in which the seeds were sown directly into the substrate, growth differences were statistically significant on all variants (table 16). The growth rate was also improved by the Xseed treatment (table 17).

Six days since sowing (3 days from sprouting), the strains height was 7mm for the control variants and between 15mm (V2-C1, chemically treated seeds) and 23mm (V4-C3, untreated seeds). The treatment is shown as improving plant height growth, even 3 days from sprouting, which would lead to the shortening of time for transplantation (in the case of obtaining seedlings by frequent seeding and transplanting), especially when concerning obtaining seedlings for greenhouse crops or early field crops. The average plant growth rates were higher than Variant 1 - control in the case of all variants treated with Xseed (table 19): between 1.6mm/day and 1.73mm/day for the controls and up to 2.8mm/day for V2-C1 - untreated seeds. Compared with V1-control, plant height growth rates were statistically significant.

The Xseed treated plants sown in peat exhibited superior height (table 21), an observation confirmed by the statistical interpretation (table 22).

Plant growth rates were higher for plants treated with Xseed. The differences were statistically significant for some variants (table 23).

Table 3

The germination of tomato seeds - seeds immersed for 60 minutes before sowing

Variant	Seeds germinated 3 days since sowing **16.07.10**	Seeds germinated 6 days since sowing **19.07.10**	Seeds germinated 8 days since sowing **21.7. 2010**	Seeds germinated 15 days since sowing **28.07.2010**	Difference in no. of seeds germinated from 16.07 to 19.07	Percent of seeds germinated from 19 to 21.07 compared to control	Difference in no. of seeds germinated 16.07 to 21.07.2010	Percent of seeds germinated from 16.07 to 21.07 compared to control	Largest percent of seeds germinate la 28.07 - compared to control
	pcs. seeds	pcs. seeds	pcs. seeds	pcs. seeds	pcs. seeds	%	pcs. seeds	%	%
V_1 Ctl - untreated seeds	**7**	**12**	**78**	**90**	**5**	**22,0**	**71**	**100,0**	**100,0**
V_2 - C1	21	73	95	95	52	7,3	74	104,2	105,6
V_3 - C2	17	82	92	92	65	3,3	75	105,6	102,2
V_4 - C3	15	87	94	94	72	2,3	79	111,3	104,4
V_1 Ctl – chemically treated seeds	**0**	**18**	**65**	**92**	**18**	**15,7**	**65**	**100,0**	**100,0**
V_2 - C1	18	81	94	94	63	4,3	76	116,9	102,2
V_3 - C2	16	87	91	91	71	1,3	75	115,4	98,9
V_4 - C3	16	83	95	95	67	4,0	79	121,5	103,3

Table 4

Summary of results: number of seeds germinated - treated and untreated variants
19.07.2010 (3 days from treatment)

Number of seeds germinated; chemically untreated seeds

VARIANT	No. of germinated seeds (pcs)	DIFFERENCE (pcs)	DIFFERENCE (%)	SIGNIF
V(0) AVG	63.50	51.50	529.17	***
V(1)	12.00	0.00	100.00	Ctl
V(2)	73.00	61.00	608.33	***
V(3)	82.00	70.00	683.33	***
V(4)	87.00	75.00	725.00	***

DL5% = 0.980 DL5% in % = 8.1667
DL1% = 1.490 DL1% in % = 12.4167
DL01% = 2.370 DL01% in %= 19.7500

Number of seeds germinated; chemically treated seeds

VARIANT	No. of germinated seeds (pcs)	DIFFERENCE (pcs)	DIFFERENCE (%)	SIGNIF
V(0) AVG	67.25	49.25	373.61	***
V(1)	18.00	0.00	100.00	Ctl
V(2)	81.00	63.00	450.00	***
V(3)	87.00	69.00	483.33	***
V(4)	83.00	65.00	461.11	***

DL5% = 1.970 DL5% in % = 10.9444
DL1% = 2.980 DL1% in % = 16.5556
DL01% = 4.750 DL01% in %= 26.3889

Table 5

Summary of results: number of seeds germinated - treated and untreated variants
21.07.2010 (6 days from treatment)

Number of seeds germinated; chemically untreated seeds				
VARIANT	No. of germinated seeds (buc)	DIFFERENCE (buc)	(%)	SIGNIF
V(0) AVERAGE	89.75	11.75	115.06	***
V(1)	78.00	0.00	100.00	Ctl
V(2)	95.00	17.00	121.79	***
V(3)	92.00	14.00	117.95	***
V(4)	94.00	16.00	120.51	***

DL5% = 2.050 DL5% in % = 2.6282
DL1% = 3.100 DL1% in % = 3.9744
DL01% = 4.940 DL01% in %= 6.3333

Number of seeds germinated; chemically treated seeds				
VARIANT	No. of germinated seeds (pcs)	DIFFERENCE (pcs)	(%)	SIGNIF
V(0) AVERAGE	86.25	21.25	132.69	***
V(1)	65.00	0.00	100.00	Ctl
V(2)	94.00	29.00	144.62	***
V(3)	91.00	26.00	140.00	***
V(4)	95.00	30.00	146.15	***

DL5% = 1.700 DL5% in % = 2.6154
DL1% = 2.580 DL1% in % = 3.9692
DL01% = 4.110 DL01% in %= 6.3231

Table 6

Tomato seed germination - sown in peat substrate and wetted with Xseed

Variant (substrate wetted with Xseed)	Seeds germinated 3 days since sowing 19.07.2010	Seeds germinated 6 days since sowing 21.7. 2010	Seeds germinated 12 days since sowing 28.07.2010	Difference in no. of seeds germinated 19.07 to 21.07 2010	Difference in no. of seeds germinated 19.07. to 21.07. 2010 compared to V1-control	Percentage of seeds germinated on 21.07.2010 compared to V1-control	Percentage of seeds germinated on 28.07 compared to V1-control
	pcs. seeds	pcs. seeds	pcs. seeds	pcs. seeds	%	%	%
Chemically untreated seeds							
V_1 Mt	21	43	89	22	100	100,0	100,0
V_2 - C1	33	83	94	50	157,1	193,0	105,6
V_3 - C2	32	87	93	55	152,4	202,3	104,5
V_4 - C3	85	95	95	10	404,8	220,9	106,7
Chemically treated seeds							
V_1 Mt	36	53	87	22	100,0	100,0	100,0
V_2 - C1	45	67	96	50	129,4	126,4	110,3
V_3 - C2	44	81	95	55	217,6	152,8	109,2
V_4 - C3	68	87	96	10	111,8	164,2	110,3

Table 7

Summary of seed germination - chemically treated and not treated seeds - peat substrate
19.07.2010 (6 days after treatment)

Number of seeds germinated; chemically untreated seeds				
VARIANT	No. germinated seeds (pcs)	DIFFERENCE (pcs)	(%)	SIGNIF
V(0) AVG	42.75	21.75	203.57	***
V(1)	21.00	0.00	100.00	Ctl
V(2)	33.00	12.00	157.14	***
V(3)	32.00	11.00	152.38	***
V(4)	85.00	64.00	404.76	***

DL5% = 1.130 DL5% in % = 5.3810
DL1% = 1.720 DL1% in % = 8.1905
DL01% = 2.740 DL01% in %= 13.0476

Number of seeds germinated; chemically treated seeds				
VARIANT	No. germinated seeds (pcs)	DIFFERENCE (pcs)	(%)	SIGNIF
V(0) AVG	48.25	12.25	134.03	***
V(1)	36.00	0.00	100.00	Ctl
V(2)	45.00	9.00	125.00	***
V(3)	44.00	8.00	122.22	***
V(4)	68.00	32.00	188.89	***

DL5% = 1.500 DL5% in % = 4.1667
DL1% = 2.270 DL1% in % = 6.3056
DL01% = 3.630 DL01% in %= 10.0833

Table 8

Summary of seed germination - chemically treated and not treated seeds - peat substrate
21.07.2010 (6 days after treatment)

Number of seeds germinated; chemically untreated seeds				
VARIANT	No. germinated seeds (pcs)	DIFFERENCE (pcs)	(%)	SIGNIF
V(0) AVG	76.92	34.25	180.27	***
V(1)	42.67	0.00	100.00	Ctl
V(2)	83.00	40.33	194.53	***
V(3)	87.00	44.33	203.91	***
V(4)	95.00	52.33	222.66	***

DL5% = 1.700 DL5% in % = 3.9844
DL1% = 2.580 DL1% in % = 6.0469
DL01% = 4.110 DL01% in %= 9.6328

Number of seeds germinated; chemically treated seeds				
VARIANTA	No. germinated seeds (pcs)	DIFERENTA (pcs)	(%)	SIGNIF
V(0) AVG	72.00	19.00	135.85	***
V(1)	53.00	0.00	100.00	Ctl
V(2)	67.00	14.00	126.42	***
V(3)	81.00	28.00	152.83	***
V(4)	87.00	34.00	164.15	***

DL5% = 0.980 DL5% in % = 1.8491
DL1% = 1.490 DL1% in % = 2.8113
DL01% = 2.370 DL01% in %= 4.4717

Table 9

Summary of seed germination - chemically treated and not treated seeds - peat substrate
28.07.2010 (6 days after treatment)

Number of seeds germinated; chemically untreated seeds					Number of seeds germinated; chemically treated seeds				
VARIANT	No. germinated seeds (pcs)	DIFFERENCE (pcs)	(%)	SIGNIF	VARIANT	No. germinated seeds (pcs)	DIFFERENCE (pcs)	(%)	SIGNIF
V(0) AVG	92.75	3.75	104.21	**	V(0) AVG	93.50	6.50	107.47	***
V(1)	89.00	0.00	100.00	Ctl	V(1)	87.00	0.00	100.00	Ctl
V(2)	94.00	5.00	105.62	***	V(2)	96.00	9.00	110.34	***
V(3)	93.00	4.00	104.49	**	V(3)	95.00	8.00	109.20	***
V(4)	95.00	6.00	106.74	***	V(4)	96.00	9.00	110.34	***
DL5% = 1.700	DL5% in % = 1.9101				DL5% = 1.970	DL5% in % = 2.2644			
DL1% = 2.580	DL1% in % = 2.8989				DL1% = 2.980	DL1% in % = 3.4253			
DL01% = 4.110	DL01% in %= 4.6180				DL01% = 4.750	DL01% in %= 5.4598			

Table 10

Root growth for experimental variants
- seeds submerged for 60 minutes before sowing - filter paper substrate

Variant	Root length at date:				Difference in growth from 16.0 to 19.07 - mm	Difference in growth from 19.0 to 21.07 - mm	Difference in growth from 16.0 to 21.07	Difference in growth from 16.0 to 28.07 -	Average growth rate
	16.07.2010	19.07.2010	21.07.2010	28.07.2010					
	mm	mm	mm	mm	mm	mm	mm	mm	mm/day
Chemically untreated seeds									
V_1 Control	2,0	8,0	16,0	18	6,00	8,00	14,0	2,00	1,2
V_2 - C1	**5,0**	**17,0**	**23,0**	26	12,00	6,00	18,0	3,00	1,73
V_3 - C2	**5,0**	**18,0**	**22,0**	27	13,00	4,00	17,0	5,00	1,80
V_4 - C3	**1,0**	**25,0**	**28,0**	31	24,00	3,00	27,0	3,00	2,07
Chemically treated seeds									
V_1 Mt	**3,0**	**12,0**	**16,0**	20	9,00	4,00	13,0	4,00	1,33
V_2 - C1	**4,0**	**14,0**	**22,0**	32	10,00	8,00	18,0	10,00	2,13
V_3 - C2	**4,0**	**16,0**	**21,0**	31	12,00	5,00	17,0	10,00	2,07
V_4 - C3	**4,0**	**16,0**	**19,0**	30	12,00	3,00	15,0	11,00	2,00

Fig. 7.

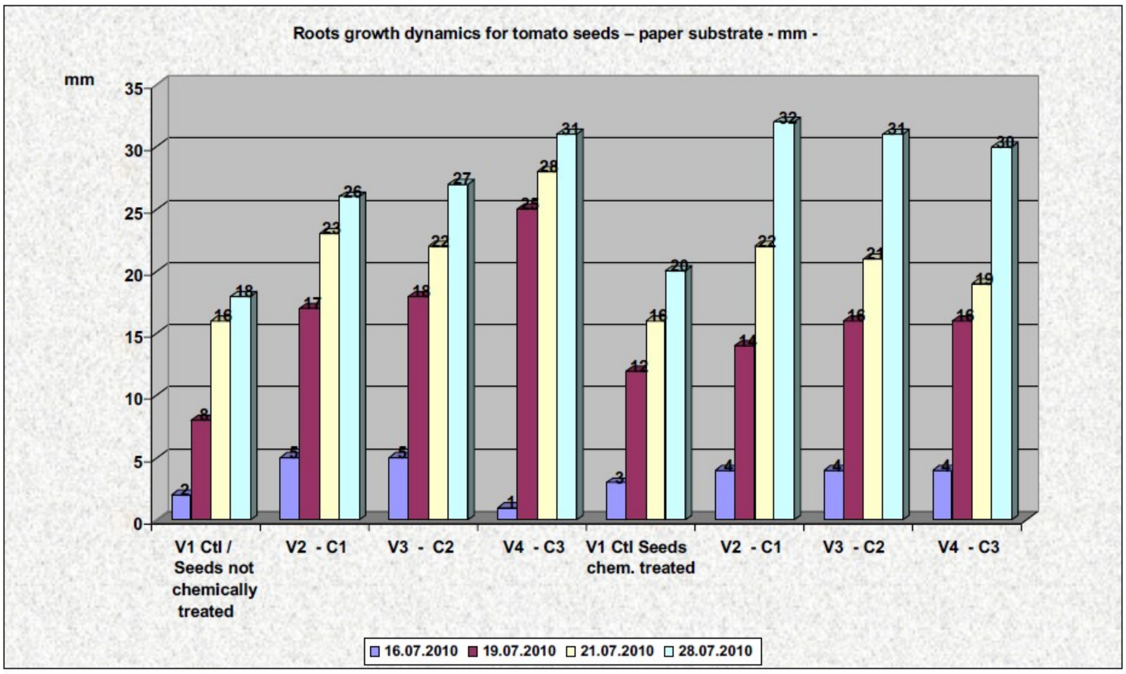


Fig. 8.

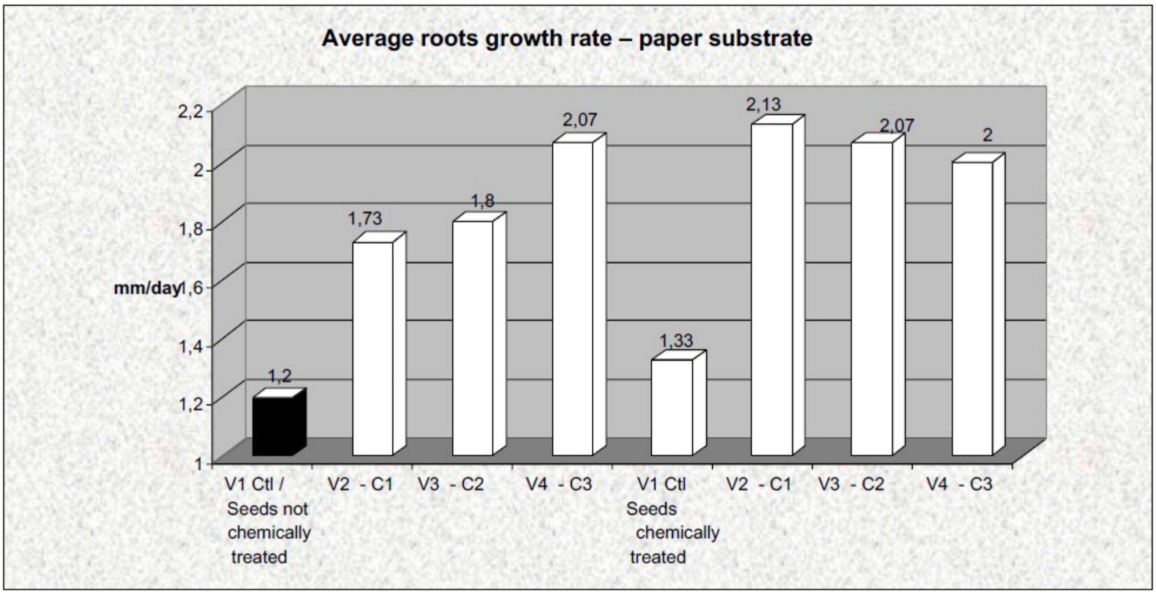


Table 11

Summary - roots growth for chemically treated and untreated variants
16.07.2010 (3 days after treatment) - paper substrate

Roots growth - seeds chemically untreated					Roots growth - chemically treated seeds				
VARIANT	Roots (mm)	DIFFERENCE (mm)	(%)	SIGNIF	VARIANT	Roots (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) AVG	3.25	1.25	162.50	N	V(0) AVG	3.75	0.75	125.00	N
V(1)	2.00	0.00	100.00	Ctl	V(1)	3.00	0.00	100.00	Ctl
V(2)	5.00	3.00	250.00	**	V(2)	4.00	1.00	133.33	N
V(3)	5.00	3.00	250.00	**	V(3)	4.00	1.00	133.33	N
V(4)	1.00	-1.00	50.00	N	V(4)	4.00	1.00	133.33	N
DL5% =	1.880	DL5% in % =	94.0000		DL5% =	2.050	DL5% in % =	68.3333	
DL1% =	2.850	DL1% in % =	142.5000		DL1% =	3.100	DL1% in % =	103.3333	
DL01% =	4.550	DL01% in %=	227.5000		DL01% =	4.940	DL01% in %=	164.6667	

Table 12

Summary - root growth for chemically treated and untreated variants
19.07.2010 (3 days after treatment) - paper substrate

Radicle growth - seeds chemically untreated					Radicle growth - chemically treated seeds				
VARIANT	Roots (mm)	DIFFERENCE (mm)	(%)	SIGNIF	VARIANT	Roots (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) AVG	17.00	9.00	212.50	***	V(0) AVG	14.50	2.50	120.83	*
V(1)	8.00	0.00	100.00	Ctl	V(1)	12.00	0.00	100.00	Ctl
V(2)	17.00	9.00	212.50	***	V(2)	14.00	2.00	116.67	*
V(3)	18.00	10.00	225.00	***	V(3)	16.00	4.00	133.33	**
V(4)	25.00	17.00	312.50	***	V(4)	16.00	4.00	133.33	**
DL5% =	1.500	DL5% in % =	18.7500		DL5% =	1.970	DL5% in % =	16.4167	
DL1% =	2.270	DL1% in % =	28.3750		DL1% =	2.980	DL1% in % =	24.8333	
DL01% =	3.630	DL01% in %=	45.3750		DL01% =	4.750	DL01% in %=	39.5833	

Table 13

Summary - roots growth for chemically treated and untreated variants
21.07.2010 (8 days after treatment) - paper substrate

Radicle growth - seeds chemically untreated					Radicle growth - chemically treated seeds				
VARIANT	Radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF	VARIANTA	Radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) AVG	22.00	7.00	146.67	***	V(0) AVG	19.25	4.25	128.33	***
V(1)	15.00	0.00	100.00	Ctl	V(1)	15.00	0.00	100.00	Ctl
V(2)	23.00	8.00	153.33	***	V(2)	22.00	7.00	146.67	***
V(3)	22.00	7.00	146.67	***	V(3)	21.00	6.00	140.00	***
V(4)	28.00	13.00	186.67	***	V(4)	19.00	4.00	126.67	***
DL5% =	0.980	DL5% in % =	6.5333		DL5% =	0.980	DL5% in % =	6.5333	
DL1% =	1.490	DL1% in % =	9.9333		DL1% =	1.490	DL1% in % =	9.9333	
DL01% =	2.370	DL01% in %=	15.8000		DL01% =	2.370	DL01% in %=	15.8000	

Table 14

Summary - radicle growth for chemically treated and untreated variants 28.07.2010 (15 days after treatment) - paper substrate

Radicle growth - seeds chemically untreated					Radicle growth - chemically treated seeds				
VARIANT	Radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF	VARIANT	Radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) AVG	25.50	7.50	141.67	***	V(0) AVG	28.25	8.25	141.25	***
V(1)	18.00	0.00	100.00	Ctl	V(1)	20.00	0.00	100.00	Ctl
V(2)	26.00	8.00	144.44	***	V(2)	32.00	12.00	160.00	***
V(3)	27.00	9.00	150.00	***	V(3)	31.00	11.00	155.00	***
V(4)	31.00	13.00	172.22	***	V(4)	30.00	10.00	150.00	***
DL5% = 2.200	DL5% in % = 12.2222				DL5% = 2.050	DL5% in % = 10.2500			
DL1% = 3.330	DL1% in % = 18.5000				DL1% = 3.100	DL1% in % = 15.5000			
DL01% = 5.310	DL01% in %= 29.5000				DL01% = 4.940	DL01% in %= 24.7000			

Table 15

Summary - radicle growth rate for chemically treated and untreated variants of tomato seeds 28.07.2010 (12 days from treatment) - paper substrate

Radicle growth - seeds chemically untreated					Radicle growth - chemically treated seeds				
VARIANT	Growth rate (mm)	DIFFERENCE (mm)	(%)	SIGNIF	VARIANT	Growth rate (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) AVG	1.70	0.50	141.67	***	V(0) AVG	1.87	0.54	140.91	N
V(1)	1.20	0.00	100.00	Ctl	V(1)	1.33	0.00	100.00	Ctl
V(2)	1.73	0.53	144.17	***	V(2)	2.10	0.77	157.64	N
V(3)	1.80	0.60	150.00	***	V(3)	2.07	0.74	155.64	N
V(4)	2.07	0.87	172.50	***	V(4)	2.00	0.67	150.38	N
DL5% = 0.180	DL5% in % = 15.0000				DL5% = 1.030	DL5% in % = 77.4436			
DL1% = 0.280	DL1% in % = 23.3333				DL1% = 1.570	DL1% in % = 118.0451			
DL01% = 0.450	DL01% in %= 37.5000				DL01% = 2.500	DL01% in %= 187.9699			

Table 16

Radicle growth for experimental variants sown in peat substrate

Variant	Radicle length mm 28.07.2010	Average growth rate
Untreated tomato seeds		
V_1 Mt	33	2,8
V_2 - C1	38	3,2
V_3 - C2	36	3,0
V_4 - C3	30	2,5
Treated tomato seeds		
V_1 Ctl	26	2,17
V_2 - C1	31	2,58
V_3 - C2	34	2,83
V_4 - C3	33	2,75

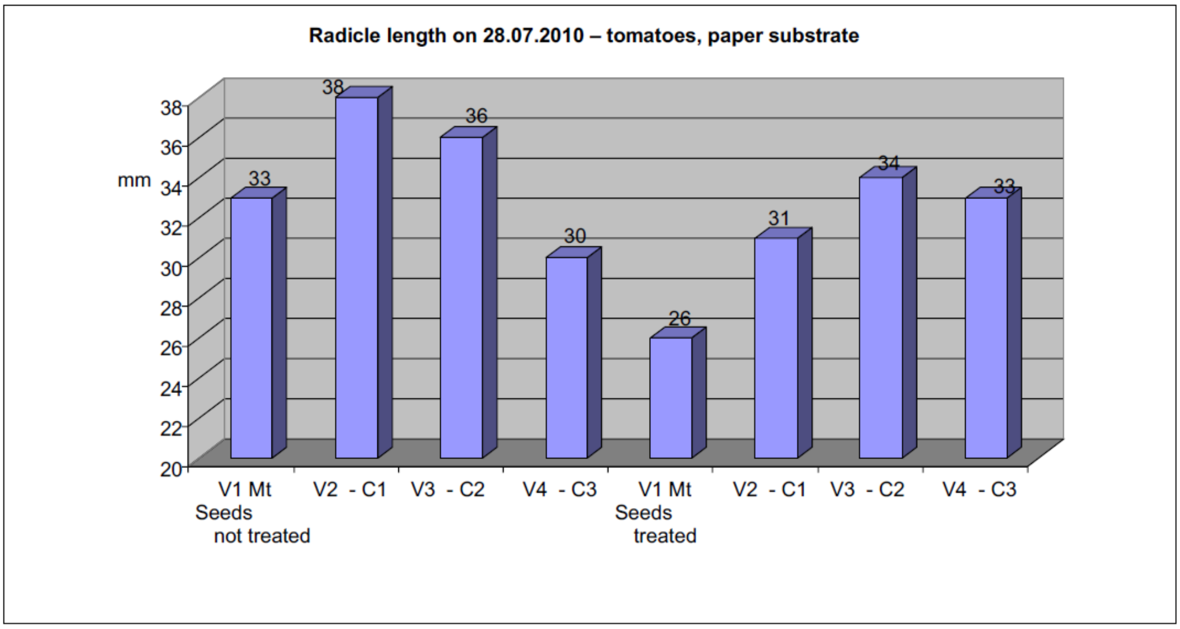


Fig. 10.

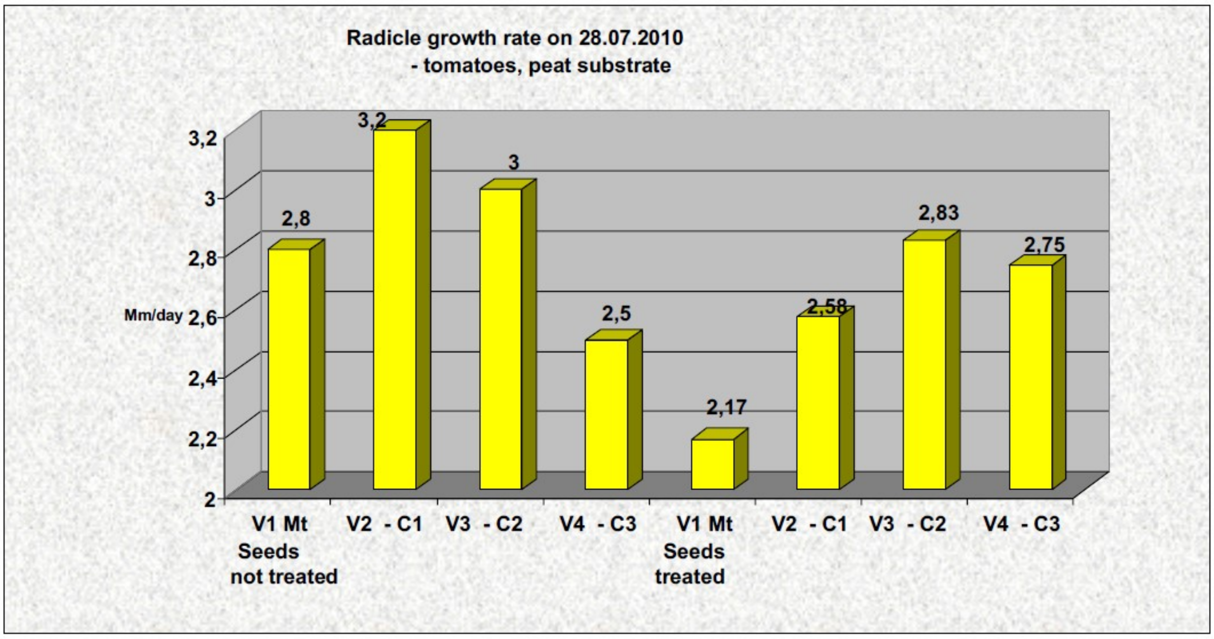


Table 17

Summary - radicle growth for chemically treated and untreated seeds 28.07.2010 (12 days from treatment) - seeds treated on peat substrate

Radicle length - seeds chemically untreated					Radicle length - chemically treated seeds				
VARIANT	Radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF	VARIANT	Radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) AVG	34.25	1.25	103.79	N	V(0) AVG	31.00	5.00	119.23	***
V(1)	33.00	0.00	100.00	Ctl	V(1)	26.00	0.00	100.00	Ctl
V(2)	38.00	5.00	115.15	***	V(2)	31.00	5.00	119.23	***
V(3)	36.00	3.00	109.09	*	V(3)	34.00	8.00	130.77	***
V(4)	30.00	-3.00	90.91	O	V(4)	33.00	7.00	126.92	***
DL5% =	2.050	DL5% in % =	6.2121		DL5% =	0.980	DL5% in % =	3.7692	
DL1% =	3.100	DL1% in % =	9.3939		DL1% =	1.490	DL1% in % =	5.7308	
DL01% =	4.940	DL01% in %=	14.9697		DL01% =	2.370	DL01% in %=	9.1154	

Table 18

Summary - radicle growth for chemically treated and untreated seeds 28.07.2010 (12 days from treatment) - seeds treated on peat substrate

Radicle length - seeds chemically untreated					Radicle length - chemically treated seeds				
VARIANT	Growth rate (mm)	DIFFERENCE (mm)	(%)	SIGNIF	VARIANT	Growth rate (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) AVG	2.88	0.08	102.68	N	V(0) AVG	2.58	0.41	119.01	***
V(1)	2.80	0.00	100.00	Ctl	V(1)	2.17	0.00	100.00	Ctl
V(2)	3.20	0.40	114.29	**	V(2)	2.58	0.41	118.89	***
V(3)	3.00	0.20	107.14	*	V(3)	2.83	0.66	130.41	***
V(4)	2.50	-0.30	89.29	OO	V(4)	2.75	0.58	126.73	***
DL5% =	0.190	DL5% in % =	6.7857		DL5% =	0.170	DL5% in % =	7.8341	
DL1% =	0.290	DL1% in % =	10.3571		DL1% =	0.250	DL1% in % =	11.5207	
DL01% =	0.470	DL01% in %=	16.7857		DL01% =	0.410	DL01% in %=	18.8940	

Table 19

Growth of tomato stemlet strains
(experimental variants - paper substrate)

Variant	Height of stemlet on date:				Difference in growth from 19.07 to 28.07 -	Average growth rate
	16.07.2010	19.07.2010	21.07.2010	28.07.2010		
	mm	mm	mm	mm	mm	mm/zi
Seed chemically untreated						
V_1 Mt		7,0	18,0	26	11,00	1,73
V_2 - C1		17,0	31,0	42	14,00	2,80
V_3 - C2		21,0	36,0	40	15,00	2,67
V_4 - C3		23,0	37,0	39	14,00	2,60
Seed chemically treated						
V_1 Mt		7,0	16,0	24	9,00	1,60
V_2 - C1		15,0	26,0	41	11,00	2,73
V_3 - C2		18,0	23,0	37	5,00	2,47
V_4 - C3		17,0	21,0	36	4,00	2,40

Table 20

Summary - seedling height of chemically treated and untreated seed variants
28.07.2010 (15 days from treatment) - paper substrate

Seedling growth - seeds chemically untreated

```
                 (mm)      (mm)    (%)
--------------------------------------------------
V( 0 ) AVG       36.75    10.75   141.35    ***
V( 1 )           26.00     0.00   100.00    Ctl
V( 2 )           42.00    16.00   161.54    ***
V( 3 )           40.00    14.00   153.85    ***
V( 4 )           39.00    13.00   150.00    ***
--------------------------------------------------
DL5% =     1.700    DL5% in % =     6.5385
DL1% =     2.580    DL1% in % =     9.9231
DL01% =    4.110    DL01% in %=    15.8077
```

Seedling growth - seeds chemically untreated

```
                 (mm)      (mm)    (%)
--------------------------------------------------
V( 0 ) AVG       34.50    10.50   143.75    ***
V( 1 )           24.00     0.00   100.00    Ctl
V( 2 )           41.00    17.00   170.83    ***
V( 3 )           37.00    13.00   154.17    ***
V( 4 )           36.00    12.00   150.00    ***
--------------------------------------------------
DL5% =     1.700    DL5% in % =     7.0833
DL1% =     2.580    DL1% in % =    10.7500
DL01% =    4.110    DL01% in %=    17.1250
```

Table 21

Average strains growth for experimental variants - seeds sown in peat substrate

Variant	**Stemlet height** mm	Average growth rate
	28.07.2010	
Seeds chemically untreated		
V_1 Mt	75	8,33
V_2 - C1	95	10,56
V_3 - C2	76	8,47
V_4 - C3	73	8,11
Chemically treated seeds		
V_1 Mt	71	7,89
V_2 - C1	87	9,67
V_3 - C2	86	9,56
V_4 - C3	84	9,33

Fig. 13.

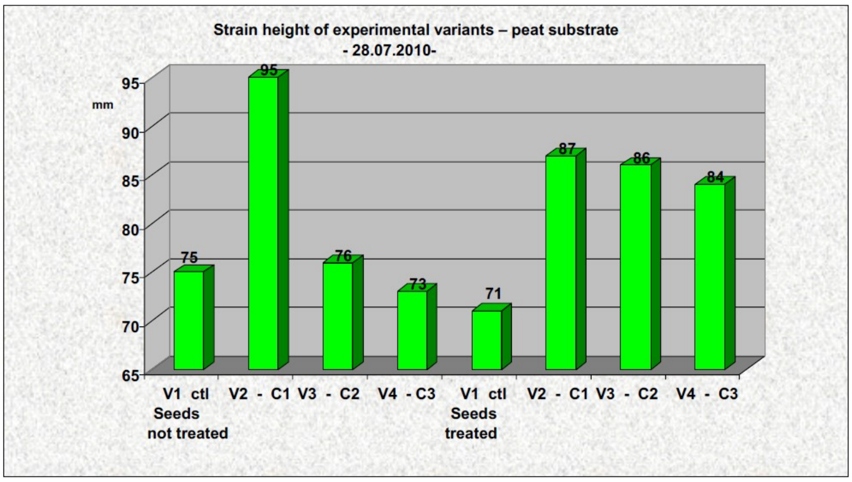


Fig. 14.

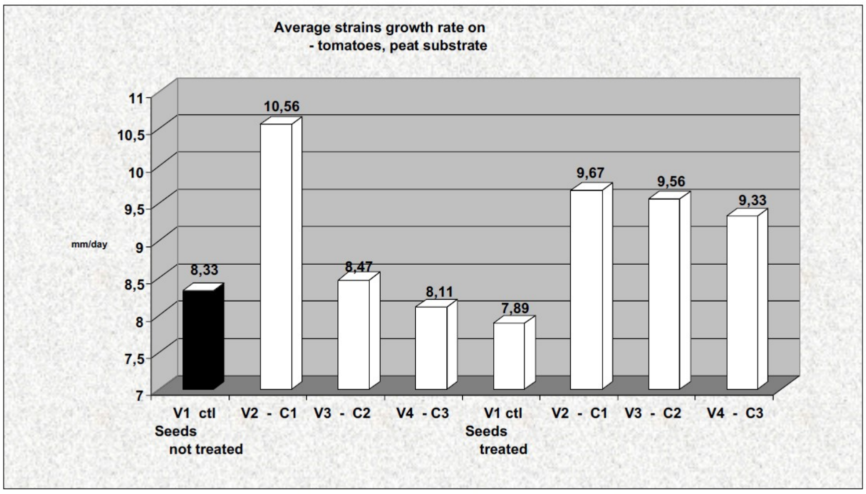


Table 22

Summary - seedling height of chemically treated and untreated seed variants
28.07.2010 (15 days after treatment)

Seedling growth - seeds chemically untreated					Seedling growth - seeds chemically untreated				
VARIANT	Height (mm)	DIFFERENCE (mm)	SIGNIF (%)		VARIANT	Height (mm)	DIFFERENCE (mm)	SIGNIF (%)	
V(0) AVG	79.75	4.75	106.33	***	V(0) AVG	82.00	11.00	115.49	***
V(1)	75.00	0.00	100.00	Ctl	V(1)	71.00	0.00	100.00	Ctl
V(2)	95.00	20.00	126.67	***	V(2)	87.00	16.00	122.54	***
V(3)	76.00	1.00	101.33	*	V(3)	86.00	15.00	121.13	***
V(4)	73.00	-2.00	97.33	OO	V(4)	84.00	13.00	118.31	***
DL5% =	0.980	DL5% in % =	1.3067		DL5% =	0.980	DL5% in % =	1.3803	
DL1% =	1.490	DL1% in % =	1.9867		DL1% =	1.490	DL1% in % =	2.0986	
DL01% =	2.370	DL01% in %=	3.1600		DL01% =	2.370	DL01% in %=	3.3380	

Table 23

Summary - seedling height growth rate of chemically treated and untreated tomato seed variants
28.07.2010 (12 days after treatment) - peat substrate

Height growth rate for seedlings of chemically untreated seeds					Height growth rate for seedlings of chemically untreated seeds				
VARIANT	Growth rate (mm/day)	DIFFERENCE (mm/day)	SIGNIF (%)		VARIANT	Growth rate (mm/day)	DIFFERENCE (mm/day)	SIGNIF (%)	
V(0) AVG	8.87	0.54	106.46	**	V(0) AVG	9.11	1.22	115.49	***
V(1)	8.33	0.00	100.00	Ctl	V(1)	7.89	0.00	100.00	Ctl
V(2)	10.56	2.23	126.77	***	V(2)	9.67	1.78	122.56	***
V(3)	8.47	0.14	101.72	N	V(3)	9.56	1.67	121.17	***
V(4)	8.11	-0.22	97.36	N	V(4)	9.33	1.44	118.25	***
DL5% =	0.230	DL5% in % =	2.7611		DL5% =	0.170	DL5% in % =	2.1546	
DL1% =	0.350	DL1% in % =	4.2017		DL1% =	0.250	DL1% in % =	3.1686	
DL01% =	0.560	DL01% in %=	6.7227		DL01% =	0.410	DL01% in %=	5.1965	

CONCLUSIONS

Xseed favourably affected the germinative energy of tomato seeds.
Statistically significant effects on seed germination were recorded three days since sowing. The percentage of germinated seeds was between 15% for V4 to 21% for V2.

The control variants has the lowest number of germinated seeds in the case of chemically untreated seeds, with just 7%, which is approximately 3 times lower than some of the variants treated with Xseed and 21 times lower than chemically treated seeds.

Using Xseed shortened the germination period of the tomato seeds by approx. 6 days (in the case of C3 concentration Xseed), and the percentage of germinated seeds was of 85% after 3 days, growing to 95% after 6 days.

Humectation of chemically treated seeds with Xseed did not affect germination.
Treating seeds with Xseed can be performed at the same time with other pesticides, thus leading to no supplemental costs per application.

Seedling producers can get seedlings 6 days earlier by using Xseed.
Tomato seeds germinated very well, in both application variants, humectated for 60 minutes prior to sowing, and humectation of the substrate after sowing. Humectation after sowing can be done by producers of cell pallets along with the germination wetting, without additional steps.

The radicular system was noted to be more vigorous when Xseed was used.
Plant height was greater for variants treated with Xseed (up to 42mm for V2-C1), when compared to the control variants. Treated variants exhibited almost double the height.
Plant growth rate was higher for plants treated with Xseed, the difference being statistically significant for some of the variants.

The time to seedling was longer when not treating the seeds prior to sowing or after by approximately 6 days.

Plant vigour was greater for variants treated with the Xseed compound.
Increasing the concentration of Xseed when soaking the seeds did not negatively affect tomato seed germination

Eggplant Seed Research

REPORT FOR EGGPLANT SEEDS
Two study variants were used in order to analyse the effect of the Xseed product on eggplant seeds.

Treatment method variants
Experiment I. Humectation for 60 minutes prior to the sowing of the chemically untreated bell pepper seeds with Xseed in various concentrations
V1 – Control Group – seeds dampened in distilled water;
V2 – Seeds dampened in solution with a concentration of C1 Xseed;
V3 - Seeds dampened in solution with a concentration of C2 Xseed;
V4 - Seeds dampened in solution with a concentration of C3 Xseed;
Seeds dampened on 13.07.2010

Experiment II. Eggplant seeds sown into a peat substratum and then dampened with Xseed solution in various concentrations:
V1 – Control Group – seeds dampened in distilled water;
V2 – Seeds dampened in solution with a concentration of C1 Xseed;
V3 - Seeds dampened in solution with a concentration of C2 Xseed;
V4 - Seeds dampened in solution with a concentration of C3 Xseed;

Seeds sown on 16.07.2010
The germination was tested in a germinator at 30 degrees Celsius during the daytime and 20 degrees Celsius during the night-time, in conformity with the germination determination standard for eggplant seeds. Data was retrieved after 6 days for the registration of the germination energy and for the final data after 14 days.

The following was determined:
- The number of germinated seeds after 3, 6, 8 and 15 days;
- The length of the roots;
- The height of the stems;
- The growth rhythm of the roots and stems
- The statistical interpretation of the results using the variance analysis

2
RESULTS OBTAINED
Upon analysing the seed germination we noted that 3 days after sowing V4 – C3 produced the highest amount of germinated seeds. 6 days after sowing all the treated variants presented a germination percentage between 62% (V3 – C2) and 80% (V4 – C4). This is a very positive result considering that eggplant seeds have quite a long germination period. Practically, 6 days after sowing the seeds germinated at a percentage of 70,45% (V3 – C2) and 85,61% (V4 – C3) as per the final date of retrieving data, when the germination was at its maximum. From 21.07.2010, after 8 days, the seeds germinated in a high percentage for the treated variants, between 72,09% for V3 and 85,11% for V4.

V4 – C3 presented the maximum percentage of germination on the 21st, practically 7 days earlier compared to V1 – the control group (see Tables 1 and 2).
Statistically there is a significant difference between the percentages of germinated seeds 6 days after sowing (see Table 3).

In the case of treated seeds directly onto the peat substratum, they germinated at a percentage of over 56% for variants 2, 3 and 4, 6 days after sowing. This is 9 days earlier (until 28.07.2010). V1, the control group registered only 10% of germination (Table 4). The seed germination 12 days after sowing presented insignificant differences from a statistical point of view. Differences distinctly significant regarding the germination energy (6 days after sowing) are highlighted (Table 5).
Also, in the case of this treatment alternative, the germinated seed percentage 6 days after sowing was of over 62% for V4 and 88,37% for V3, compared to 21.07.2010, 8 days after sowing. The fact that also in this alternative treatment, the germinated seed percentage was superior to the V1–control group leads to quality saplings being produced 9 days earlier (Table 6).

For the experimental treatment in which seeds were dampened 60 minutes prior to being sown, notable statistical differences were seen concerning radicle growth. All variants showed a radicle growth larger than that of the untreated control group, as early as 19.07., 8 days after sowing. These differences were maintained even after 8 days (21.07.2010) and after 15 days of sowing (28.07.2010).

The radicle growth rhythm was highest for those seeds that were dampened for 60 minutes prior to being sown (see Tables 8 and 9).
In the case of variants sown directly into the substratum and dampened with the Xseed product it is to be noted that on 28.07, 12 days after sowing, the radicles presented a much higher growth than the control group. These were in between 34 mm for V4 – C3 and 37 mm for V3 – C2. From a statistical point of view, the differences between the control group and the treated variants were significant. This is favourable as the development of a vigorous and fast radicular system leads to obtaining an appropriate sapling in a shorter timeframe (Table 10).

On 28.07.2010, the eggplant seedlings presented heights between 18 mm for the V1 control group and
48 mm for V4 – C3. All treated variants presented bigger height growth than the control group. The percentage of growth is significant: 211,11% for V2 and 266,67% for V4 (Tables 11 and 12).
3 Height growth was also notable on the stemlets of the eggplant seedlings. The height of the seedlings on the peat variant that was treated direct was between 8mm for V2 – C1 and 26mm for V3 – C2. Percentagewise that is 17,78% and 57,78% over V1, the control group. Statistically, the differences between the treated variants and the control group were distinctly significant, which stresses the effect of the treatments with the Xseed product (see Table 13).

Table 1

Eggplant seed germination (50 seeds per repetition)

Variant	Germinated seeds 3 days after sowing 16.07.10		Germinated seeds 6 days after sowing 19.07.10		Germinated seeds 8 days after sowing 21.7. 2010		Percentage of germinated seeds - compared to the control group	Germinated seeds 15 days after sowing 28.07.2010		Percentage of germinated seeds - compared to the control group
	Seed pieces	%	Seed pieces	%	Seed pieces	%	%	Seed pieces	%	%
V_1 Ctrl Gr	0	0	6	12	31	62	100,0	42	84	100,0
V_2 - C1	3	6	38	76	46	92	148,4	46	92	109,5
V_3 - C2	0	0	31	62	43	86	138,7	44	88	104,8
V_4 - C3	8	16	40	80	47	94	151,6	47	94	111,9

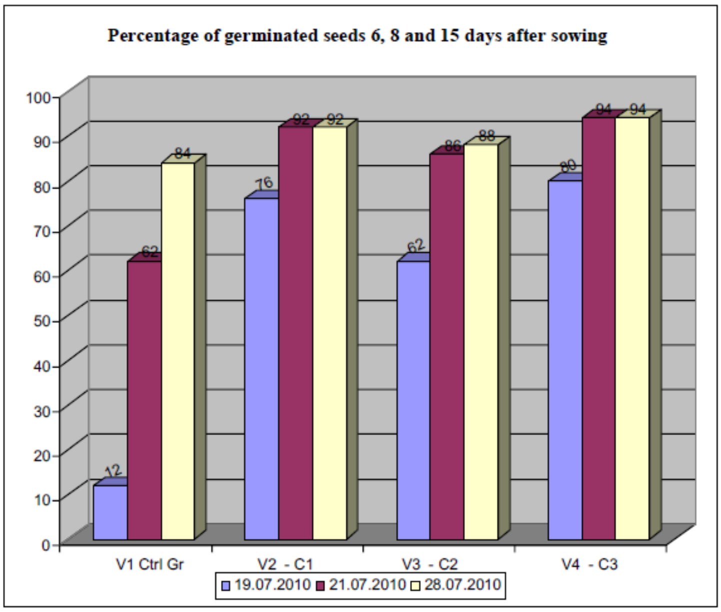


Table 2

Percentage of germination on 19.07.2010 compared to the final germination from 28.07.2010

Variant	Percentage of germination on 19.07.2010 (after 6 days) compared to the germination percentage on 21.07.2010 (after 8 days)	Percentage of germination on 19.07.2010 (after 6 days) compared to the final germination percentage on 28.07.2010 (after 12 days)
V_1 - Ctrl Gr	19,35	14,29
V_2 - C1	82,61	82,61
V_3 - C2	72,09	70,45
V_4 - C3	85,11	85,11

Table 3

The summary of the seed germination

Seed germination on 19.07.2010

VARIANT	Germination (%)	DIFFERENCE (%)	(%)	SIGNIF
V(0) average	57.50	45.50	479.17	***
V(1)	12.00	0.00	100.00	Ctrl Gr
V(2)	76.00	64.00	633.33	***
V(3)	62.00	50.00	516.67	***
V(4)	80.00	68.00	666.67	***

DL5% = 0.980 DL5% in % = 8.1667
DL1%= 1.490 DL1% in % = 12.4167
DL01% = 2.370 DL01% in %= 19.7500

Seed germination on 21.07.2010

VARIANT	Germination (%)	DIFFERENCE (%)	(%)	SIGNIF
V(0) average	83.50	21.50	134.68	**
V(1)	62.00	0.00	100.00	Ctrl Gr
V(2)	92.00	30.00	148.39	***
V(3)	86.00	24.00	138.71	**
V(4)	94.00	32.00	151.61	***

DL5% = 10.420 DL5% in % = 16.8065
DL1%= 15.760 DL1% in % = 25.4194
DL01% = 25.120 DL01% in %= 40.5161

Seed germination on 28.07.2010

VARIANT	Germination (%)	DIFFERENCE (%)	(%)	SIGNIF
V(0) average	89.50	5.50	106.55	N
V(1)	84.00	0.00	100.00	Ctrl Gr
V(2)	92.00	8.00	109.52	N
V(3)	88.00	4.00	104.76	N
V(4)	94.00	10.00	111.90	N

DL5% = 18.010 DL5% in % = 21.4405
DL1%= 27.250 DL1% in % = 32.4405
DL01% = 43.420 DL01% in %= 51.6905

Table 4

Eggplant seed – variant where seeds where sown directly into the peat substratum and then dampened with Xseed solution

Variant	Seeds germinated after 3 days 16.07.10		Seeds germinated after 6 days 19.07.10		Seeds germinated after 8 days 21.7. 2010		Percentage of germinated seeds - compared to the control group	Seeds germinated after 15 days 28.07.2010		Percentage of germinated seeds - compared to the control group
	Seed pieces	%	Seed pieces	%	Seed pieces	%	%	Seed pieces	%	%
V_1 Ctrl Gr	0	0	5	10	28	56	100,0	43	86	100,0
V_2 - C1	0	0	31	62	42	84	150,0	44	88	102,3
V_3 - C2	0	0	38	76	43	86	153,6	46	92	107,0
V_4 - C3	0	0	28	56	45	90	160,7	45	90	104,7

Table 5

The summary of results for seeds treated on the peat substratum

Seed germination on 19.07. 2010

VARIANT	Germination (%)	DIFFERENCE (%)	(%)	SIGNIF
V(0) average	51.00	41.00	510.00	***
V(1)	10.00	0.00	100.00	Ctrl Gr
V(2)	62.00	52.00	620.00	***
V(3)	76.00	66.00	760.00	***
V(4)	56.00	46.00	560.00	***

DL5% = 1.970 DL5% in % = 19.7000
DL1%= 2.980 DL1% in % = 29.8000
DL01% = 4.750 DL01% in %= 47.5000

Seed germination on 21.07. 2010

VARIANT	Germination (%)	DIFFERENCE (%)	(%)	SIGNIF
V(0) average	79.00	23.00	141.07	***
V(1)	56.00	0.00	100.00	Ctrl Gr
V(2)	84.00	28.00	150.00	***
V(3)	86.00	30.00	153.57	***
V(4)	90.00	34.00	160.71	***

DL5% = 1.750 DL5% in % = 3.1250
DL1%= 2.650 DL1% in % = 4.7321
DL01% = 4.220 DL01% in %= 7.5357

Seed germination on 28.07. 2010

VARIANT	Germination (%)	DIFFERENCE (%)	(%)	SIGNIF
V(0) average	89.00	3.00	103.49	N
V(1)	86.00	0.00	100.00	Ctrl Gr
V(2)	88.00	2.00	102.33	N
V(3)	92.00	6.00	106.98	N
V(4)	90.00	4.00	104.65	N

DL5% = 10.620 DL5% in % = 12.3488
DL1%= 16.070 DL1% in % = 18.6860
DL01% = 25.600 DL01% in %= 29.7674

Table 6

Percentage of germination on 19.07. 2010 compared to the final germination percentage on 28.07. 2010 (after 12 days)

Variant	Percentage of germination on 19.07. 2010 (after 6 days) compared to the germination percentage on 21.07. 2010 (after 8 days)	Percentage of germination on 19.07. 2010 compared to the final germination percentage on 28.07. 2010 (after 12 days)
V_1 Ctrl Gr	17,86	11,63
V_2 - C1	73,81	70,45
V_3 - C2	88,37	82,61
V_4 - C3	62,22	62,22

Table 7

Length growth of the radicle in the experiments

Variant	Length of radicle on the: 16.07. 2010	19.07. 2010	21.07. 2010	28.07. 2010	Difference in growth from 16.0 to 19.07	Difference in growth from 19.0 to 21.07 -	Difference in growth from 21 to 28.07	Difference in growth from 19.0 to 28.07 -	Average growth rhythm
	mm	mm	mm	mm	mm	mm	mm	mm	mm/day
V_1 Ctrl Gr		2	8	26	2	6	18	24	2,89
V_2 - C1		9	36	38	9	27	2	29	4,22
V_3 - C2		4	34	39	4	30	5	35	4,33
V_4 - C3		8	32	35	8	24	3	27	3,89

Table 8

Summary of results concerning the length of the radicle on the eggplant seeds

Summary of results concerning the length of the radicle on the eggplant seeds 19.07.2010

VARIANT	Length of radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) average	5.75	3.75	287.50	**
V(1)	2.00	0.00	100.00	Ctrl Gr
V(2)	9.00	7.00	450.00	***
V(3)	4.00	2.00	200.00	*
V(4)	8.00	6.00	400.00	***

DL5% = 1.700 DL5% in % = 85.0000
DL1%= 2.580 DL1%in % = 129.0000
DL01% = 4.110 DL01% in %= 205.5000

Summary of results concerning the length of the radicle on the eggplant seeds 21.07.2010

VARIANT	Length of radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) average	27.50	19.50	343.75	***
V(1)	8.00	0.00	100.00	Ctrl Gr
V(2)	36.00	28.00	450.00	***
V(3)	34.00	26.00	425.00	***
V(4)	32.00	24.00	400.00	***

DL5% = 1.700 DL5% in % = 21.2500
DL1%= 2.580 DL1%in % = 32.2500
DL01% = 4.110 DL01% in %= 51.3750

Summary of results concerning the length of the radicle on the eggplant seeds 28.07.2010

VARIANT	Length of radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) average	34.50	8.50	132.69	***
V(1)	26.00	0.00	100.00	Ctrl Gr
V(2)	38.00	12.00	146.15	***
V(3)	39.00	13.00	150.00	***
V(4)	35.00	9.00	134.62	***

DL5% = 2.050 DL5% in % = 7.8846
DL1%= 3.100 DL1%in % = 11.9231
DL01% = 4.940 DL01% in %= 19.0000

Table 9

Length growth and growth rhythm of the radicle in the peat substratum experiment

Variant	Length of radicle mm	Medium growth rhythm
	28.07.2010	mm/day
V_1 Ctrl Gr	13	1,44
V_2 - C1	36	4,00
V_3 - C2	37	4,11
V_4 - C3	34	3,78

Table 10

Summary of results regarding the growth in length and the growth rhythm of the radicle in the experiments where the seeds were sown directly into the peat substratum

Summary of results regarding the length of the radicle on 28.07. 2010

VARIANT	Length of radicle (mm)	DIFFERENCE (mm)	(%)	SIGNIF
(0) average	30.00	17.00	230.77	***
V(1)	13.00	0.00	100.00	Ctrl Gr
V(2)	36.00	23.00	276.92	***
V(3)	37.00	24.00	284.62	***
V(4)	34.00	21.00	261.54	***

DL5% = 1.700 DL5% in % = 13.0769
DL1%= 2.580 DL1%in % = 19.8462
DL01% = 4.110 DL01% in %= 31.6154

Summary of results regarding the growth rhythm of the radicle on 28.07. 2010

VARIANT	Growth rhythm (mm/day)	DIFFERENCE (mm/day)	(%)	SIGNIF
V(0) average	3.33	1.89	231.42	*
V(1)	1.44	0.00	100.00	Ctrl Gr
V(2)	4.00	2.56	277.78	**
V(3)	4.11	2.67	285.42	**
V(4)	3.78	2.34	262.50	*

DL5% = 1.610 DL5% in % = 111.8055
DL1%= 2.430 DL1%in % = 168.7500
DL01% = 3.880 DL01% in %= 269.4444

Table 11

Growth in height of the eggplant strains stemlet in the experimental alternatives

Variant	Medium height of the stemlet on the:				Difference in growth from the 21.07 to 28.07 -	Percentage compared to control group	Medium growth rhythm
	16.07.2010	19.07.2010	21.07.2010	28.07.2010			
	mm	mm	mm	mm	mm	%	mm/day
V_1 Ctrl Gr			3	18	15	100,00	2,57
V_2 - C1			32	38	6	211,11	5,43
V_3 - C2			28	34	6	188,89	4,86
V_4 - C3			41	48	7	266,67	6,86

Table 12

Summary of results regarding the height of the eggplant strains on 28.07. 2010

VARIANT	Height of stemlet (mm)	DIFFERENCE (mm)	(%)	SIGNIF
V(0) average	34.50	16.50	191.67	***
V(1)	18.00	0.00	100.00	Ctrl Gr
V(2)	38.00	20.00	211.11	***
V(3)	34.00	16.00	188.89	***
V(4)	48.00	30.00	266.67	***

DL5% = 1.700 DL5% in % = 9.4444
DL1%= 2.580 DL1% in % = 14.3333
DL01% = 4.110 DL01% in %= 22.8333

Table 13

Medium height growth of the eggplant strains where the seeds were planted directly in the peat substratum

Variant	Height of stemlet on the 28.07.2010	Difference		Signif.	Medium growth rhythm	Percentage compared to control group
	mm	(mm)	(%)		mm/day	%
V_1 Ctrl Gr	45	0.00	100.00	Ctrl Gr	5,00	100,0
V_2 - C1	53	8.00	117.78	***	5,89	117,8
V_3 - C2	71	26.00	157.78	***	7,89	157,8
V_4 - C3	65	20.00	144.44	***	7,22	144,4
	DL5% = 1.700 DL1%= 2.580 DL01% = 4.110	DL5% in % = 3.7778 DL1% in % = 5.7333 DL01% in %= 9.1333				

CONCLUSIONS

Germination was favoured, as such after 3 days V4 – C3 showed a germination percent of 16%, whilst the control group V1 showed no germination at all.

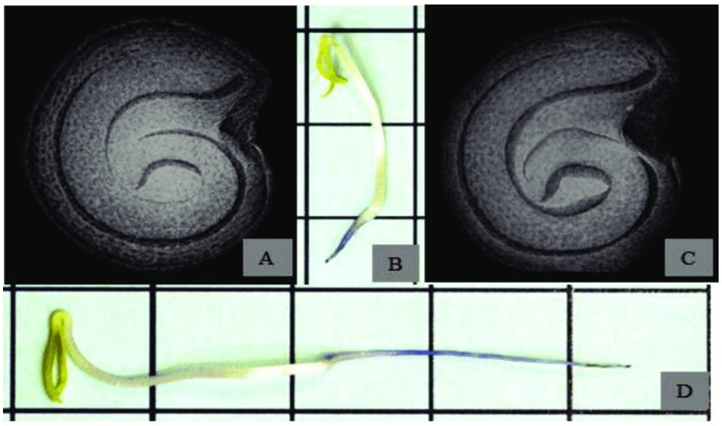
6 days after sowing all treated variants (in both methods) a germination percentage between 76% for the dampening of the peat substratum method and 80% for the prior dampening of the seeds before sowing method was visible.

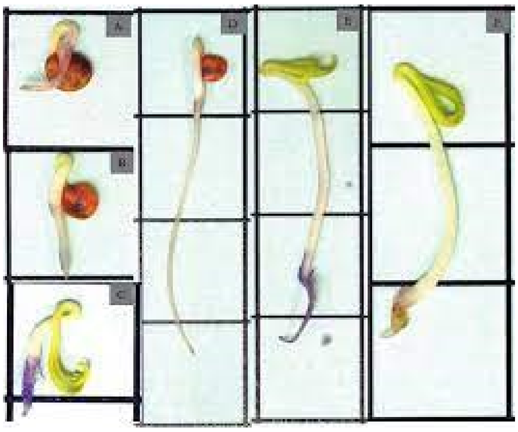
Germination time for all treated variants of the eggplant seeds was shortened by approximately 9 days, which helps obtain quality saplings earlier. Keeping in mind the species-specific Requirements regarding the germination temperature and timeframe. This provides an advantage as it saves on the energy required for heating the environment where the saplings are produced.

The forming and growth rhythm of the roots was accelerated for all the Xseed-treated variants. This is a relevant aspect as the development of a vigorous radicular system leads to obtaining an appropriate sapling.

All the Xseed-treated variants presented height growth greater than that of the V1 untreated control group: 111, 11% for V2 and 166,67% for V4.

The plants obtained through being directly sown into the peat substratum also presented significant growth, with percentages of up to 57, 78% above the control group. This aspect can be exploited when sowing directly into the cellular pallets and then dampened with the Xseed product, saving on the pricking-out labour.





Bell Pepper Seed Research

Research report on the testing of bell pepper seed germination in laboratory conditions
The influence of the bio stimulator Xseed on bell pepper seed germination
Biological material: bell pepper seeds, OPAL automatic germination chamber
Analysis method:
Three repetitions each of 50 bell pepper seeds were used in the experiment. 150 bell pepper seeds were analysed overall, in 2 different work methods.
Treatment method variants
Experiment I. The testing of bell pepper seed germination on a substratum of filtering paper
a. Submerging for 60 minutes prior to the sowing of the chemically untreated bell pepper seeds with Xseed in various concentrations:
V1 – Control – seeds submerged in distilled water;
V2 – Seeds submerged in solution with a concentration of C1 Xseed;
V3 - Seeds submerged in solution with a concentration of C2 Xseed;
V4 - Seeds submerged in solution with a concentration of C3 Xseed;
Seeds were treated on the 13.07.2010.
Experiment II. The testing of bell pepper seed germination on a substratum of peat
a. Humectation of the peat substratum after the sowing of chemically untreated bell pepper seeds with Xseed in various concentrations:
V1 - Control Group– seeds submerged in distilled water;
V2 – Seeds submergedin solution with a C1 Xseed concentration;
V3 - Seeds submerged in solution with a C2 Xseed concentration;
V4 - Seeds submerged in solution with a C3 Xseed concentration;
Seeds were treated on the 16.07.2010.
2
The germination was tested in a germinator at 30 degrees Celsius during the daytime and 20 degrees Celsius during the night-time, in conformity with the germination determination standard for bell pepper seeds.
The following was determined:
- The number of germinated seeds after 3, 6, 8 and 15 days;
- The length of the roots;
- The height of the stems;
- The growth rhythm of the roots and stems;
- The statistical interpretation of the results using the variance analysis.

To highlight the effects of the treatment the data was statistically processed.
The experiment took place in the Vegetable and Ornamental Plants Chair, at the Horticulture University in USAMC – Bucharest, during the following timeframe: 13.07 – 28.07.2010

Results

Experiment I - The testing of bell pepper seed germination on a substratum of filtering paper

Based on the data retrieved, the following was determined:

On 16.07.2010, 3 days after sowing, the lowest number of germinated bell pepper seeds was found for the 4th Variant – the C3 Xseed solution. The highest percentage of germinated bell seeds (22%) was found for the 2nd Variant – C1.

Also, from a statistical point of view, a considerable difference was notable between the germination and the control Variant (V1).

On the 19.07.2010, the highest germination percentage was registered by V4.

The percentage of seed germination was also maintained on the 21.07.2010.

Table 1

Variant	No. of germinated seeds	Germination percent %	No. of germinated seeds	Germination percent %	No. of germinated seeds	Germination percent %	No. of germinated seeds	Germination percent %
	16.07.2010		19.07.10		21.7. 2010		28.07.10	
V_1Ctrl G	7,33	15	14	28	21	42	41	82
V_2 - C1	11,0	22	27	54	43	86	46	92
V_3- C2	7,67	15	26	52	46	92	47	94
V_4 - C3	5,33	11	31	62	47	94	47	94

Three days after sowing, the seeds treated with Xseed C1 presented the highest germination percent (22%) compared to the rest of the variants. The difference was notable from a statistical point of view: 50% above the control variant. The 1st control presented a percentage of only 14,67% germinated seeds (see table 2).

The number of germinated seeds, the percentage of germinated seeds as opposed to the control group, on 16.07.2010, three days after treatment.

Species	Variant	Number of germinated seeds	Germination percent as opposed to V_1 %	Difference: Pieces	Difference: % as opposed to V1	Meaning
Bell pepper	V_1 Ctrl Gr	7,33	14,67	0,00	100,00	Ctrl Gr
	V_2 - C1	11,0	22,00	3,67	150,00	*
	V_3 - C2	7,67	15,34	0,33	104,55	N
	V_4 - C3	5,33	10,66	-2,00	72,73	N
				DL5% = 2,710 DL1% = 4,100 DL01% = 6.530	DL5% in % = 36,9545 DL1% in % = 55,9091 DL01% in %= 89.0455	

Six days after sowing the differences are distinct as opposed to the control group, the germinated seeds with V3-C1 being at 85,71% and the V4-C3 ones at 121,43% (see table 3).

Table 3

The summary of the results for the germinated seeds percentage for the 19.07.2010, 6 days after sowing

Species	Variant	Number of germinated seeds	Germination percent as opposed to V_1 %	Difference		
				Pieces	% as opposed to V1	Meaning
Bell pepper	V_1 Ctrl Gr	14	28	0.00	100.00	Ctrl Gr
	V_2 - C1	27	54	26.00	192.86	***
	V_3 - C2	26	52	24.00	185.71	***
	V_4 - C3	31	62	34.00	221.43	***
				DL5% = 1.130 DL1% = 1.720 DL01% = 2.740	DL5% in % = DL1% in % = DL01% in %=	4.0357 6.1429 9.7857

On the 21.07.2010, eight days after sowing, the percentage of germinated seeds was 94% for V4, a difference of 123,81% over the control group (V1). All the treated variants showed higher germination values than V1, with percentages of over 104% (see table 4).

5

Table 4

The summary of the results for the germinated seeds percentage for the 21.07.2010

VARIANT Germination

Percentage DIFFERENCE SIGNIF

(%) (% sem.) (%)

V(0) average 78.50 36.50 186.90 ***

V(1) 42.00 0.00 100.00 Ctrl Gr

V(2) 86.00 44.00 204.76 ***

V(3) 92.00 50.00 219.05 ***

V(4) 94.00 52.00 223.81 ***

DL5% = 1.700 DL5% in % = 4.0476

DL 1% = 2.580 DL 1% in % = 6.1429

DL 01% = 4.110 DL01% in %= 9.7857

----

15 days after sowing the differences were up to 14, 63% compared to the control group V1, which shows that the bell pepper seeds germinated in a shorter timeframe in the treated variants. The control group germinated in a longer timeframe compared to the rest of the treated variants.

Table 5

The summary of the results for the germinated seeds percentage for the 28.07.2010, 15 days after sowing.

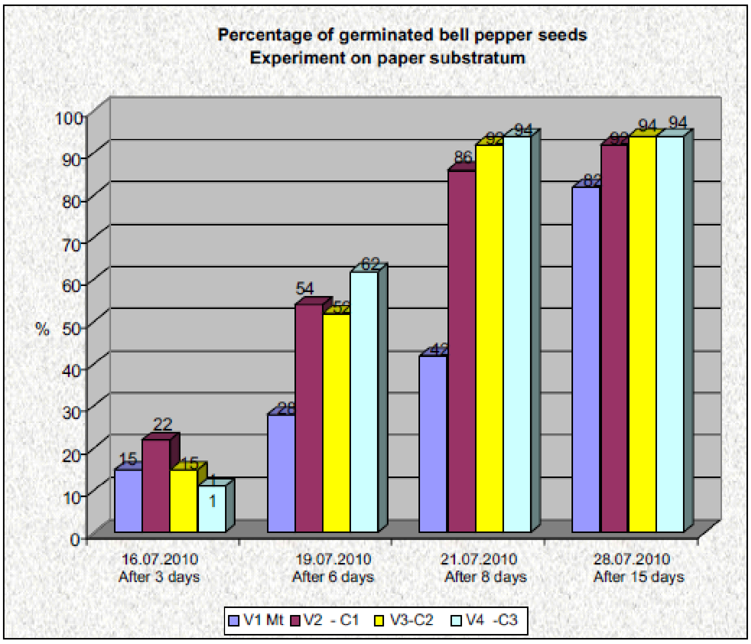
VARIANT Germination
Percentage DIFFERENCE SEMF
(%) (% sem.) (%)

V(0) average 90.50 8.50 110.37 ***
V(1) 82.00 0.00 100.00 Ctrl Gr
V(2) 92.00 10.00 112.20 ***
V(3) 94.00 12.00 114.63 ***
V(4) 94.00 12.00 114.63 ***

DL5% = 0.980 DL5% in % = 1.1951
DL1% = 1.490 DL1% in % = 1.8171
DL01% = 2.370 DL01% in %= 2.8902

The germination percent was 7% higher for V2 in comparison with the control group after 3 days. After 6 days, the percentage of germinated seeds for V4-C3 was 34% higher than that of the control group. After 8 days all the treated variants presented germination percentages of over 86%, the difference was most notabel at V4-C3.

Fig. 1



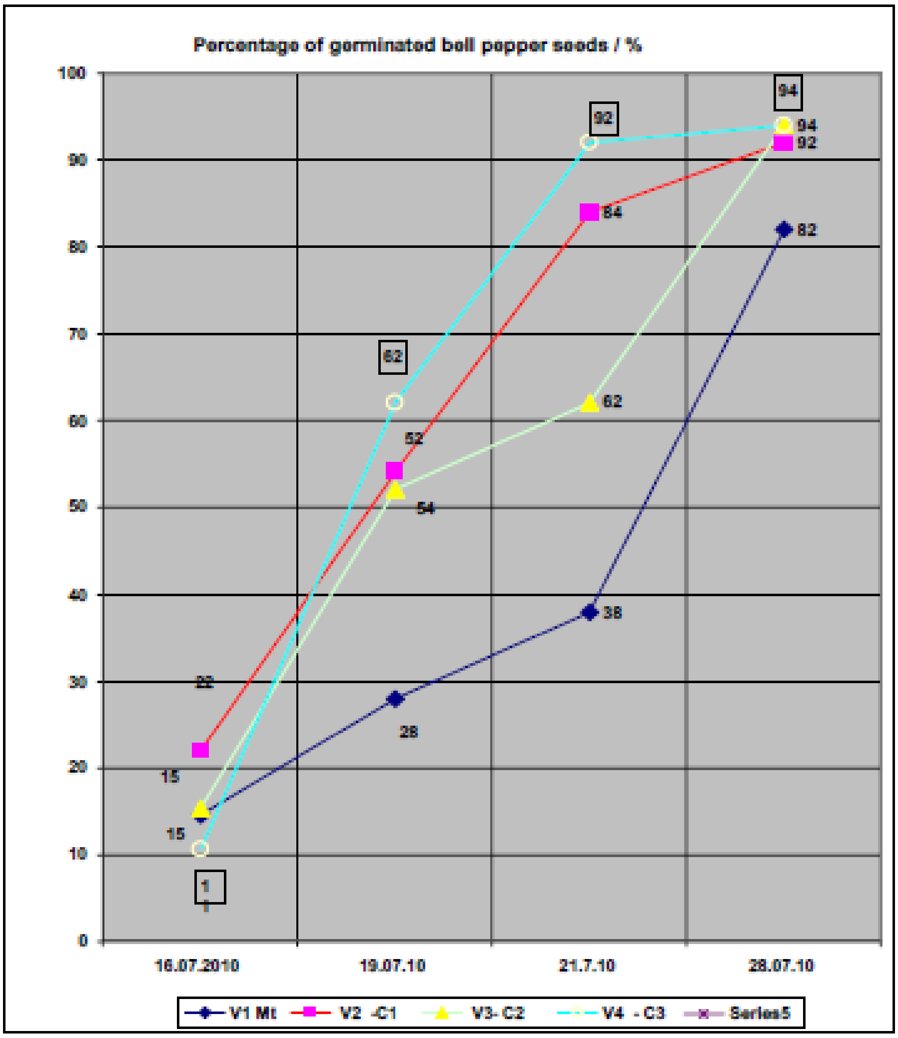
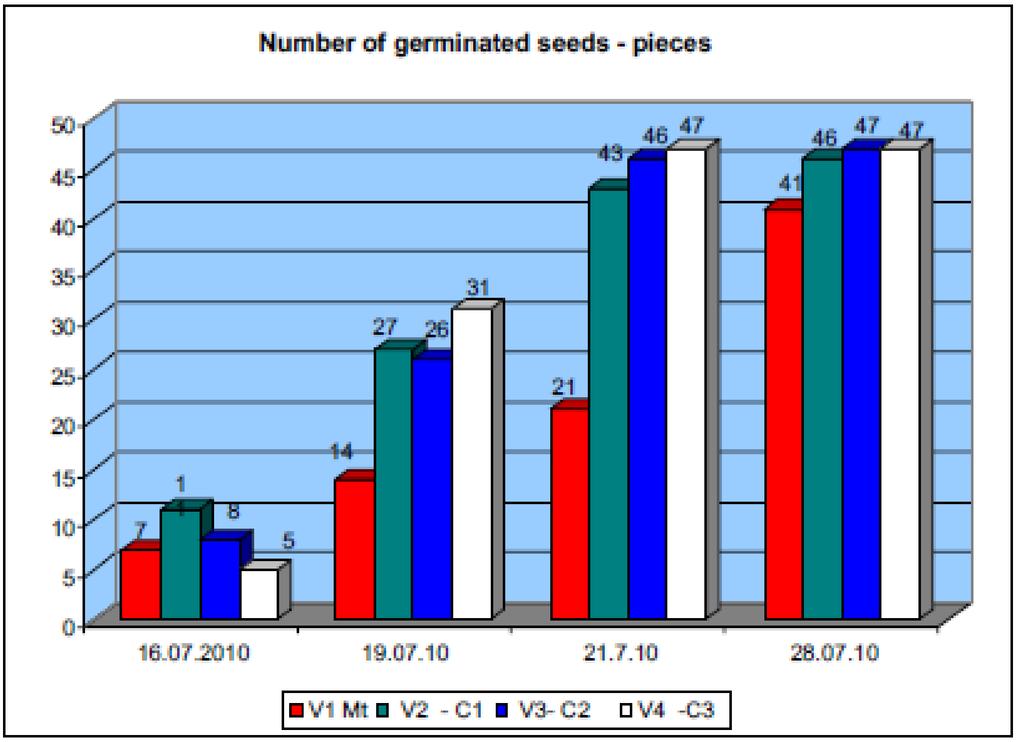

Percentage of germinated bell pepper seeds / %
100
90
80
70
60
50
40
30
20
10
0
94
92
94
92
84
82
62
62
52
54
38
22
28
15
15
1
16.07.2010
19.07.10
21.7.10
28.07.10
V1 Mt
V2 -C1
V3- C2
V4 - C3
Series5

Fig. 3


Number of germinated seeds - pieces
50
45
40
35
30
25
20
15
10
5
0
7
1
8
5
14
27
26
31
21
43
46
47
41
46
47
47
16.07.2010
19.07.10
21.7.10
28.07.10
V1 Mt
V2 - C1
V3- C2
V4 -C3

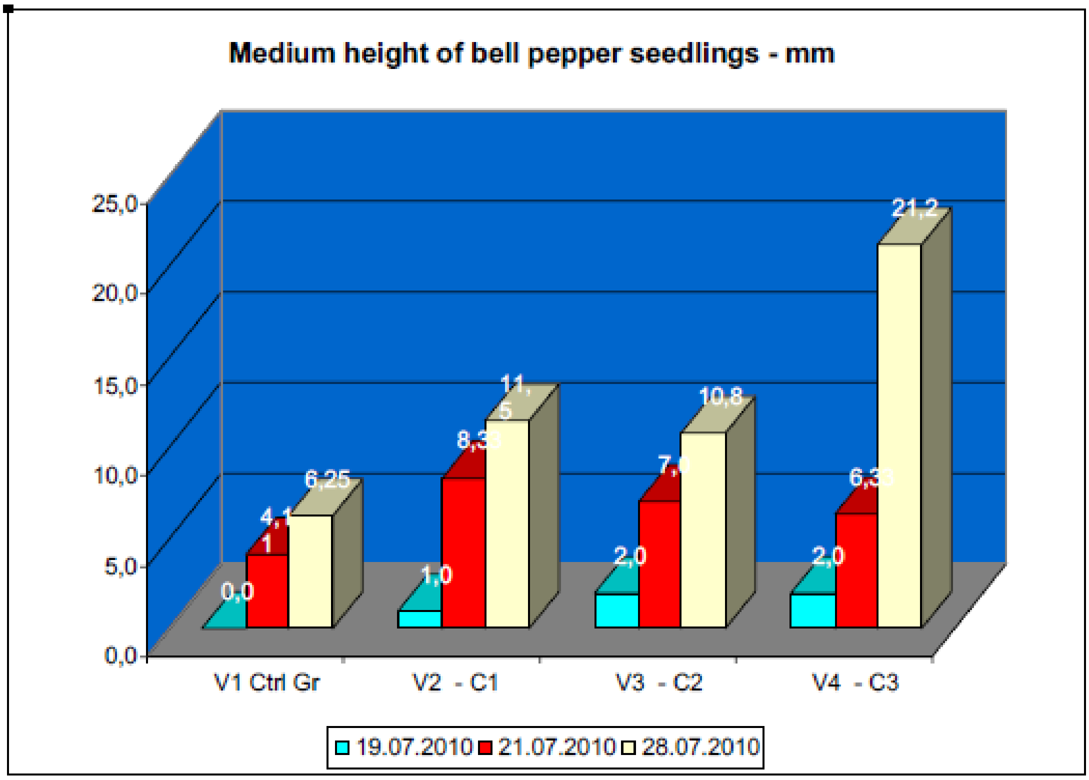


Fig 4

Bell pepper **seedlings** presented differences even after only 6 days from sowing. The highest was registered at V4.
15 days after sowing the growth of the seedling was of 21,2 mm, and from a statistical point of view the differences were **significant** as per V1 (see Table 8).

Table 6

Average height of seedlings - mm

Species	Variant	Average height of seedlings:		
		19.07.2010 At 6 days	21.07.2010 At 8 days	28.07.2010 At 15 days
Bell pepper	V_1 Ctrl Gr	0	4,11	6,25
	V_2 - C1	1	8,33	11,5
	V_3 - C2	2	6,00	10,8
	V_4 - C3	2,0	6,33	21,2

Table 7

The meaning regarding the height of bell pepper seedlings on 21.07.2010

Variant	Medium height of seedlings (mm) on the :	Difference		Significance
	21.07.2010	(mm)	(%)	
V_1 Ctrl Gr	4,11	0,00	100,00	Ctrl Gr
V_2 - C1	8,33	4,22	202,68	***
V_3 - C2	7,00	2,89	170,32	***
V_4 - C3	6,33	2,22	154,01	**
		DL5% = 0,560 DL1% = 1,040 DL01% = 2.310	DL5% in % = 13,6253 DL1% in % = 25,3041 DL01% in %= 56.2044	

Table 8

The meaning regarding the height of bell pepper seedlings on 28.07.2010

Variant	Medium height of seedlings (mm) on the 28.07.2010	Difference		Significance
	mm	mm	%	
V_1 Ctrl Gr	6.25	0.00	100.00	Ctrl Gr
V_2 - C1	11.50	5.25	184.00	***
V_3 - C2	10.80	4.55	172.80	***
V_4 - C3	21.20	14.95	339.20	***
	DL5% = 1.700 DL5% in % = 27.2000 DL1% = 2.580 DL1% in % = 41.2800 DL01% = 4.110 DL01% in %= 65.7600			

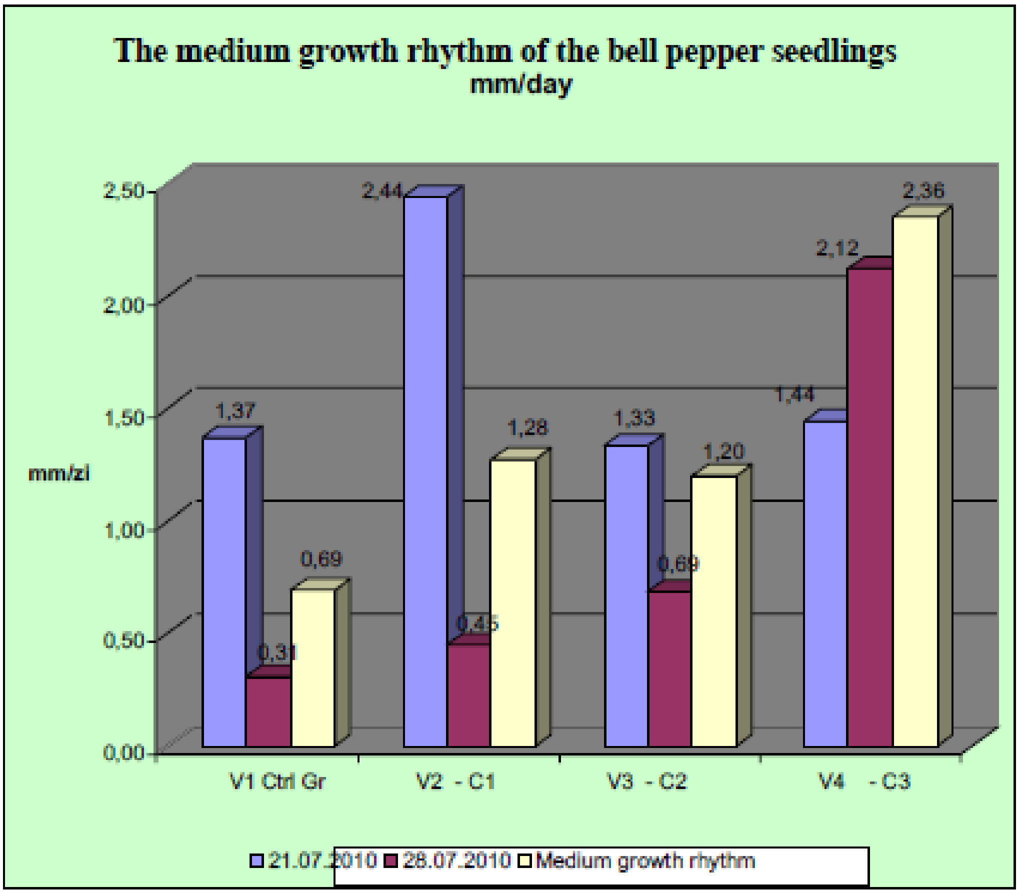
The medium growth rhythm of the seedlings was higher for the treated variants (see table 9).

Table 9

The medium growth rhythm of the seedlings

Variant	From 19.07 to the 21.07	From the 21.07 to the 28.07	Medium growth rhythm
V_1 Ctrl Gr	1,37	0,31	0,69
V_2 - C1	2,44	0,45	1,28
V_3 - C2	1,33	0,69	1,20
V_4 - C3	1,44	2,12	2,36

Fig. 5



All of the treated variants presented cotyledonous leaves bigger than the ones from V1, of 22,5 mm for V4 and 5,85 mm for V2 (see table 10, fig. 6).

Table 10

Size of cotyledonous leaves - mm

Species	**Variant**	**Length of the cotyledonous leaves - mm**	
		21.07.2010	**28.07.2010**
Bell pepper	V1 Ctrl Gr	3,25	5,85
	V2 - C1	5	12
	V3 - C2	4,87	12,5
	V4 - C3	5,00	22,5

Fig. 6

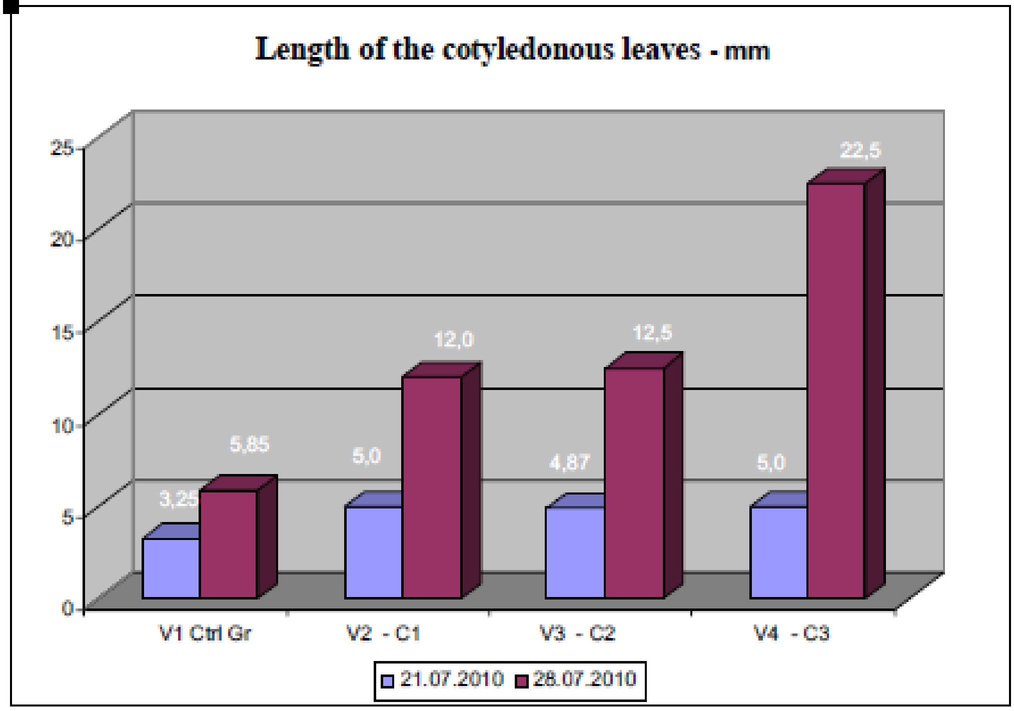


Table 11

Size of cotyledonous leaves for bell pepper seedlings on 21.07.2010

Variant	Size of cotyledonous leaves	Difference		Significance
	mm	(mm)	(%)	
V1 Ctrl Gr	3,25	0,00	100,00	Ctrl Gr
V2 - C1	6,33	3,08	194,77	***
V3 - C2	4,87	1,62	149,85	**
V4 - C3	5,00	1,75	153,85	**
		DL5% = 0,440 DL1% = 0,820 DL01% = 1,820	DL5% in % = DL1% in % = DL01% in %=	13,5385 25,2308 56,0000

From tables 10 and 11 we notice that the root of the sapling was bigger for all the treated variants: between 2 mm for V2 and 5 mm for V3 (after 6 days).
After 15 days for V3 and V4 the lengtht was 32,5 mm and 36,8 mm respectively.
The control group presented the lowest growth in height, the differences between the variants as opposed to the control group being very significative (for V3 and V4) and significative (for V2)

Table 12

Length of the root

Species	Variant	Length of the root - mm		
		19.07.2010	21.07.2010	28.07.2010
Bell pepper	V1 Ctrl Gr	1	12,66	24,33
	V2 - C1	2	24,7	28,11
	V3 - C2	5,0	25,6	32,5
	V4 - C3	2,1	26,5	36,8

Table 13

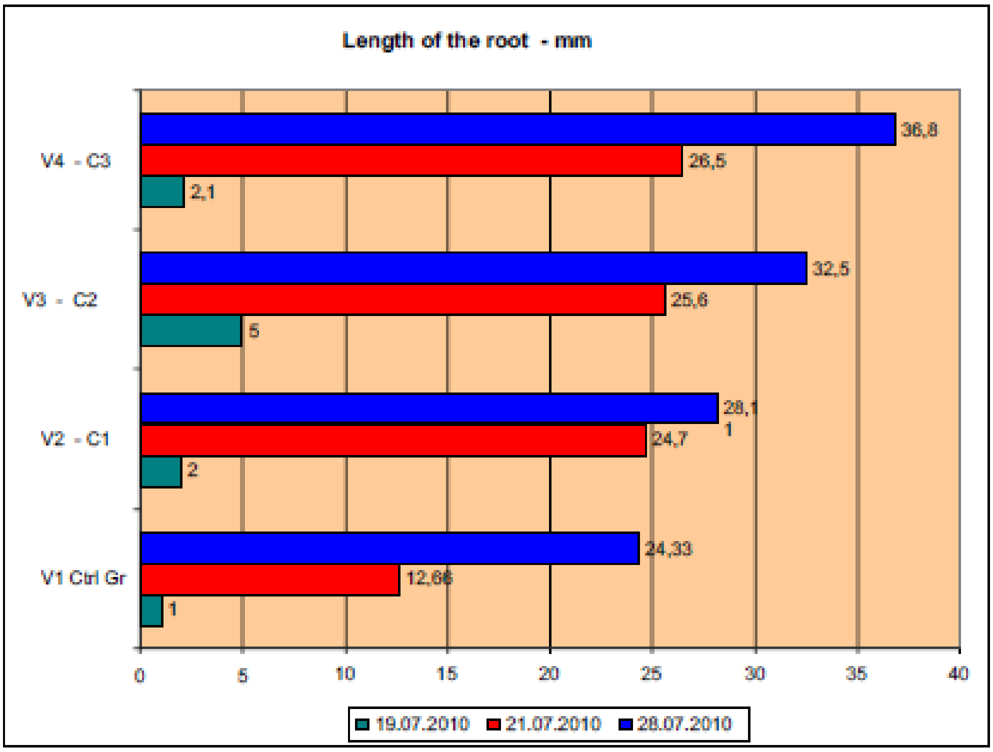
The summary of results regarding the length of the root on bell pepper seedlings

VARIANT LENGTH OF THE
ROOT DIFFERENCE SIGNIF
(mm) (mm) (%)

V(0) average 30.51 5.88 123.86 ***
V(1) 24.63 0.00 100.00 Ctrl Gr
V(2) 28.11 3.48 114.11 **
V(3) 32.50 7.87 131.94 ***
V(4) 36.80 12.17 149.39 ***

DL5% = 1.500 DL5% in % = 6.0893
DL 1% = 2.270 DL 1% in % = 9.2152
DL 01% = 3.620 DL 01% in %= 14.6955

Fig.10



Experiment II. The testing of bell pepper seed germination on a substratum of peat

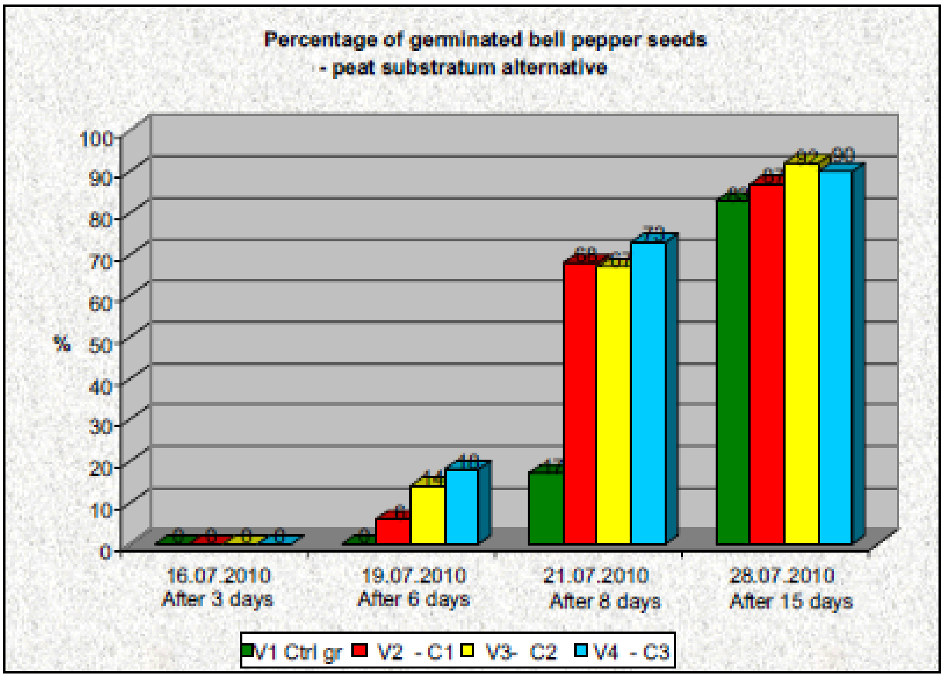
The germination percentage of the bell pepper seeds was, on 21.07. 2010, six days after sowing between 67% for V3 and 73% for V4 compared to only 17% for V1, the control group. Practically in 8 days the germination percentage for the non treated seeds was of only 20,5% and the rest of the variants of over 72,8% (V3). The highest germination percent was 81%, registered at V4, C3.

Table 12

Percentage of germination of bell pepper seeds – peat substratum alternative

Variant	Germination percentage %	Germination percentage %	Germination percentage %	Percentage of germinated seeds at 8 days compared to	Germination percentage %
	3 days after 16.07.2010	6 days after 19.07.2010 -	8 days after		After 15 days
			21.07.2010	after 15 days	28.07.2010
V_1 Ctrl Gr	0	0	17	20,5	83
V_2 - C1	0	6	68	78,2	87
V_3- C2	0	14	67	72,8	92
V_4 - C3	0	18	73	81,1	90

Fig.11



CONCLUSIONS

The percent of seeds germinated was 22% (V2) comparatively with control (V1) which only germinated 15%.

After 8 days the germination percentage for the treated variants was very close to the maximum germination percentage of the seeds after 15 days. The control group V1 was almost 50% smaller compared to the rest of the variants.

Early seed germination is preferred in order to obtain seedling in a shorter timeframe. Economically speaking, obtaining a seedling earlier also reduces costs.

Based on the obtained results the following data was extracted:
- Treated seeds presented a higher germination percentage in a shorter time period compared to the control group one – without any treatment.

- All the variants on which the treatment with Xseed was applied to for 60 minutes had a superior seedling height than that of the control group but the best results were obtained by V2 on which we applied the solution with a C1 concentration;
- Although remarkable differences can be observed for all the treated variants, we can appreciate that for bell pepper only using the V2 variant is practical.

RESULTS

Table 1

Variant	Number of germinated seeds -pcs.-	Percent germinated %	Number of seeds germinated -pcs.-	Percent germinated %	Number of seeds germinated -pcs.-	Percent germinated %
	16.07.2010		19.07.10		21.7. 2010	
V_1 Ctl	0	0	0	0	28	56
V_2 - C1	16	32	27	54	44	88
V_3- C2	8	16	27	54	43	86
V_4 - C3	40	80	46	92	47	94

Table 2

Average number of germinated seeds (50 seeds per round)

Variant	3 days since sowing	6 days since sowing	8 days since sowing	Total seeds considered each round
	16.07.2010	19.07.10	21.7.10	
V1 Ctl	0	0	28	50
V2 - C1	16	27	44	50
V3- C2	8	27	43	50
V4 - C3	40	46	47	50

Table 3

Number and percentage of germinated seeds, compared to the control 21.07.2010 (3 days after treatment)

Variant	Number of germinated seeds -pcs.-	Percentage of germinated seeds, compared to V_1 %	Difference Pcs.	Difference % compared to control	Significance
V_1 Ctl	0	28.00	0.00	100.00	Ctl
V_2 - C1	16	44.00	16.00	157.14	***
V_3 - C2	8	43.00	15.00	153.57	***
V_4 - C3	40	47.00	19.00	167.86	***
		DL5% = 1.700 DL1% = 2.580 DL01% = 4.110	DL5% in % = 6.0714 DL1% in % = 9.2143 DL01% in %= 14.6786		

Fig. 1

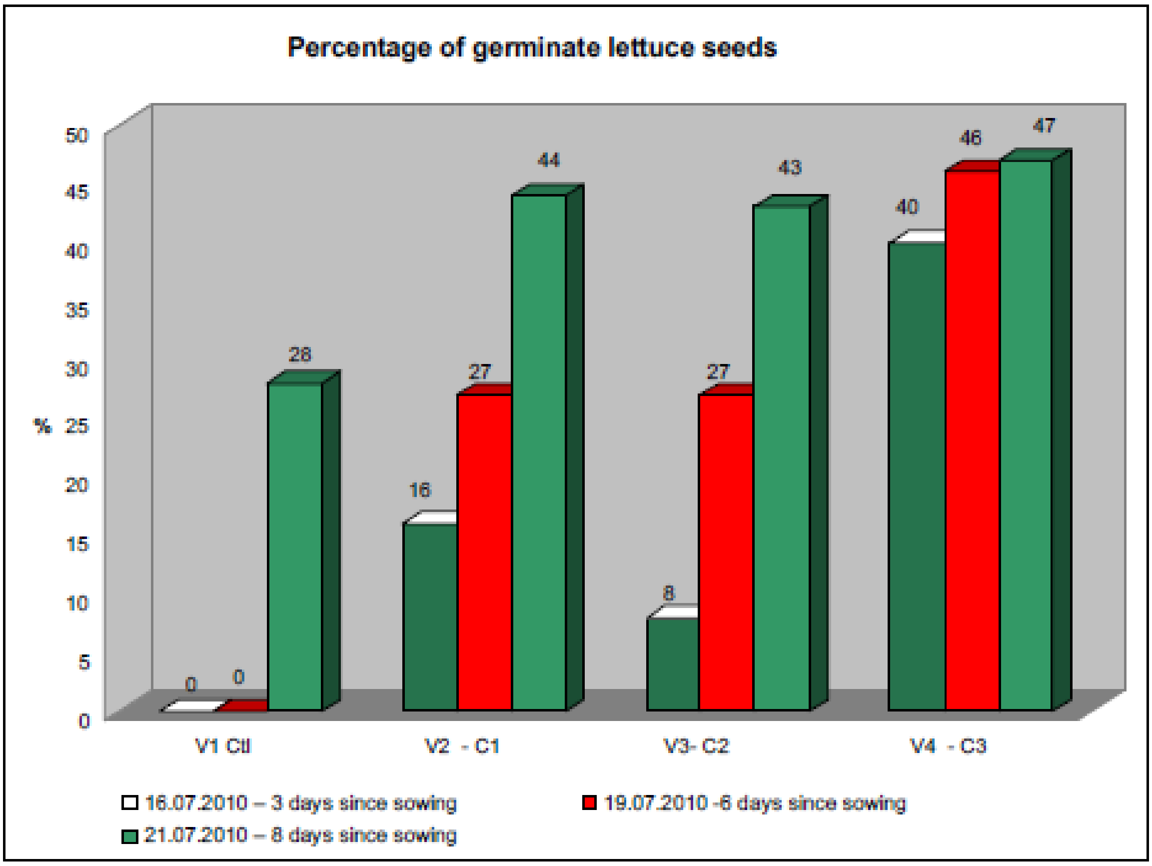
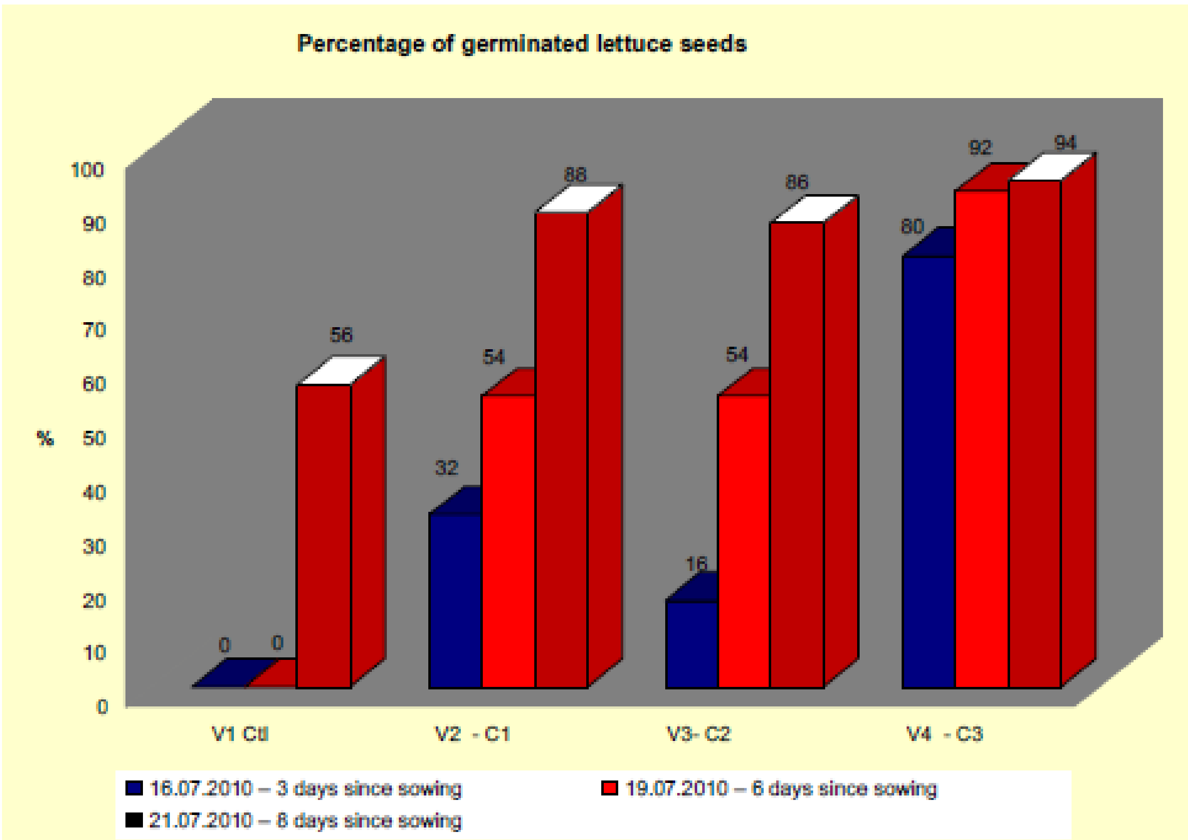

Percentage of germinate lettuce seeds
%
0
5
10
15
20
25
30
35
40
45
50
0
0
28
16
27
44
8
27
43
40
46
47
V1 Ctl
V2 - C1
V3- C2
V4 - C3
16.07.2010 – 3 days since sowing
19.07.2010 -6 days since sowing
21.07.2010 – 8 days since sowing

Fig. 2


Percentage of germinated lettuce seeds
%
0
10
20
30
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V1 Ctl
V2 - C1
V3- C2
V4 - C3
16.07.2010 – 3 days since sowing
19.07.2010 – 6 days since sowing
21.07.2010 – 8 days since sowing

Fig. 3

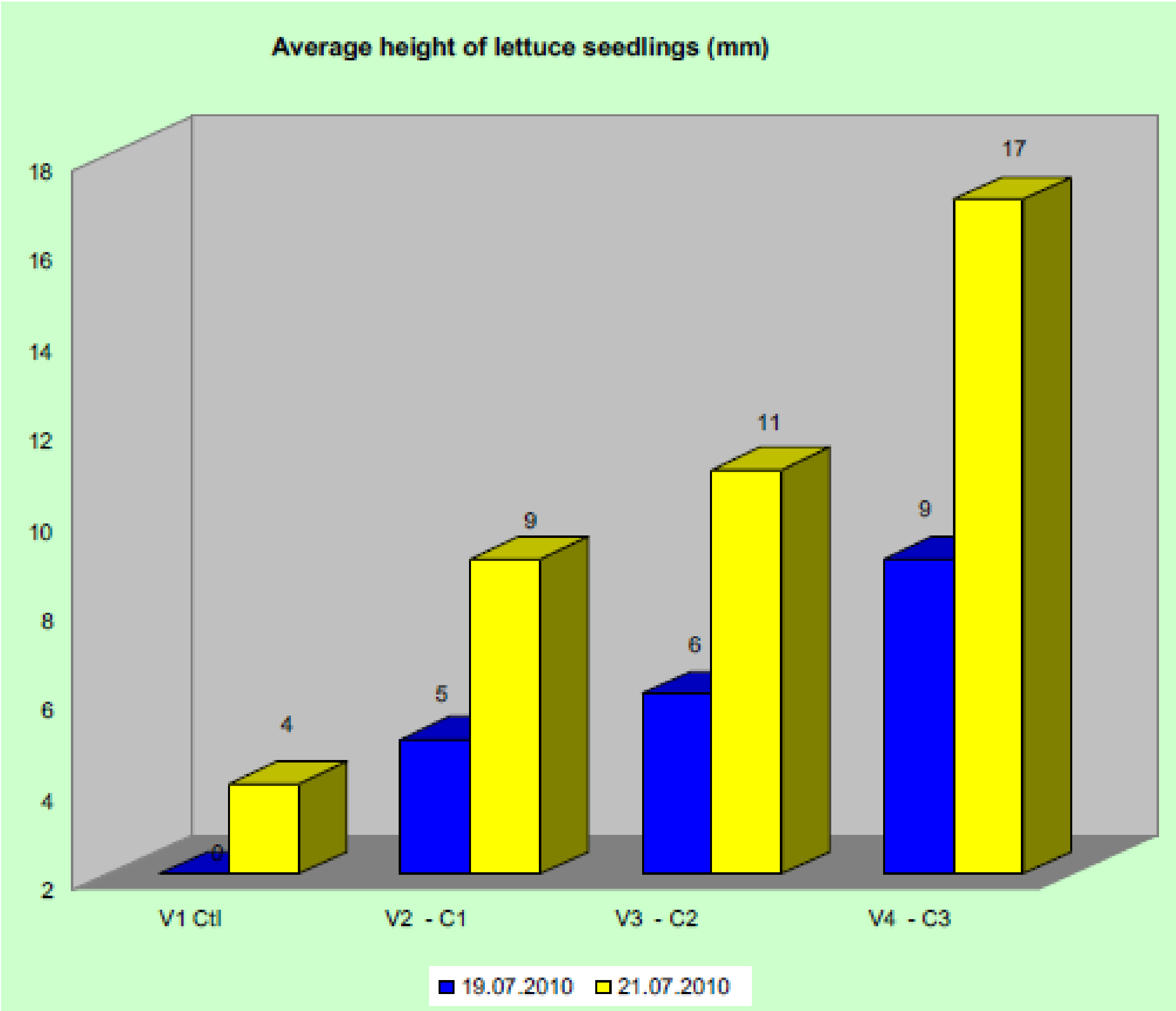

Table 4

Average height of lettuce seedlings (mm)

Variant	Average height of lettuce seedlings (mm)	
	19.07.2010	21.07.2010
V_1 Ctl		4
V_2 - C1	**5**	**9**
V_3 - C2	**6**	**11**
V_4 - C3	**9**	**17**

Table 5

Significance of lettuce plantula (seedling) height on 21.07.2010

Variant	Average height of seedlings	Difference		Significance
	21.07.2010	(mm)	(%)	
V_1 Ctl	4.00	0.00	100.00	Ctl
V_2 - C1	9.33	5.33	233.33	***
V_3 - C2	11.00	7.00	275.00	***
V_4 - C3	17.00	13.00	425.00	***
		DL5% = 1.970 DL1% = 2.980 DL01% = 4.750		DL5% in % = 49.2500 DL1% in % = 74.5000 DL01% in %= 118.7500

Species		Average seedling height (mm)			
	Variant	19.07.2010	21.07.2010		
lettuce	V_1 Ctl	0	4		
	V_2 - C1	**5**	**9**		
	V_3 - C2	**6**	**11**		
	V_4 - C3	**9**	**17**		

Table 7

Average height growth rate of seedlings

Variant	From 19.07 to 21.07.2010	Avg. growth rate
V_1 ctl	1.33	0.5
V_2 - C1	1.33	1.13
V_3 - C2	1.67	1.38
V_4 - C3	2.67	2.13


V3
13.06.20


V3
16.07.2010


V3 19.07.2010


V3 21.07.2010


V4
13.06.2010


V4
16.07.2010


V4 19.07.2010


V4 21.07.2010

Artichoke Seeds Research

THE EFFECT OF TREATMENT WITH "BIOROOTZ" ON SEED GERMINATION AND INITIAL ROOT GROWTH

<u>Experiment 1.</u>

50 seeds of *Cynara cardunculus* (cardoon) were submerged in to water for 45 minutes, whereas another 50 cynara seeds were submerged to water solution of biorootz 0.1% per volume for 45 minutes. The above seeds, plus another set of 50 cynara seeds which were not submerged to water, were all placed to a germination chamber, and their germination rate was determined daily, under temperature and humidity conditions set to 22°C and 100%, respectively.

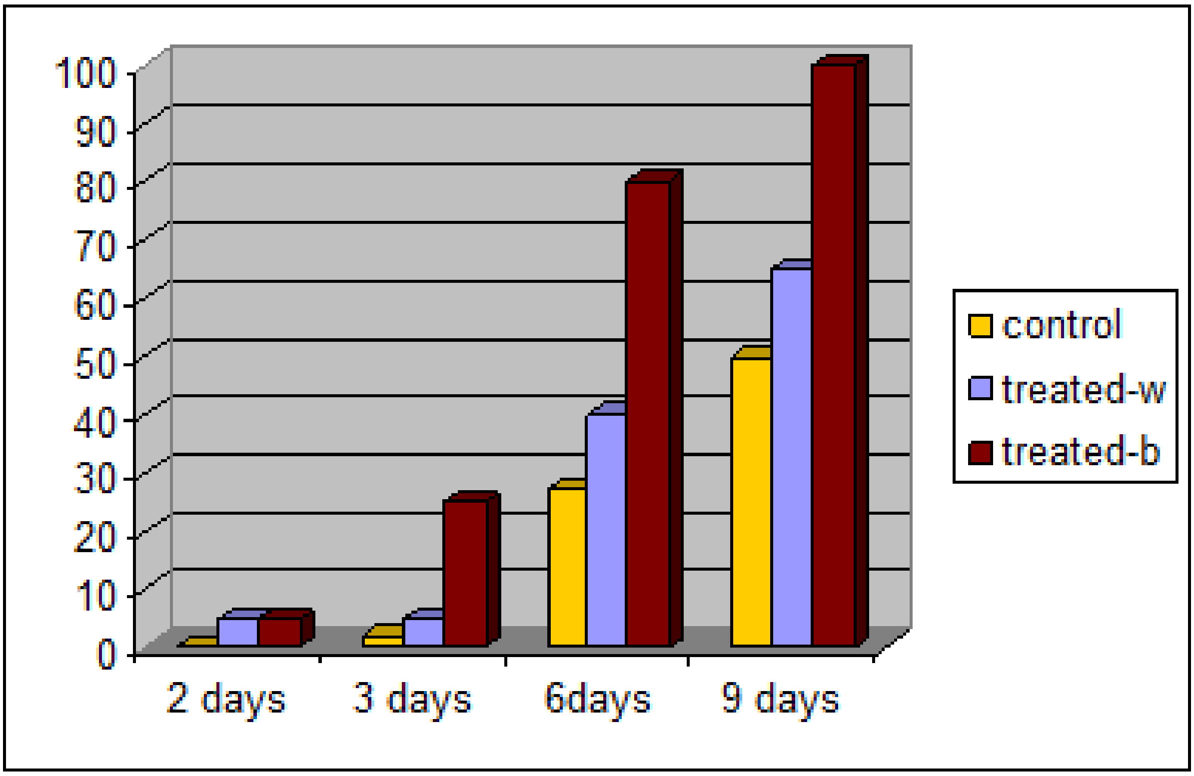

Fig. 1. The germination rate (y-axis, in %) with the days elapsed since treatment (x-axis).

Fig. 1 demonstrates that watering the seeds (treated-w and treated-b) started earlier the process of germination than in the control. Especially, under the treatment with biorootz (treated-b) this effect was remarkable.

As can be seen, 3 days after treatment, the biorootz treated seeds (treated-b) showed germination of 25% versus only 5% of the water treated (treated-w) and 2% of the control; 6 days after treatment, the biorootz treated seeds had germinated already by

80% (100% was reported one day later), twice as much as the water treated seeds and more than three times as much as the control. Even after nine days, the control seeds were germinated only by 50%, 15% lower than the treated-w seeds. Full germination (100%) was reported 11 days after the experiment initiation.

<u>Experiment 2.</u>

After the control seeds (not submerged to water – not treated) were germinated, 10 seeds were placed on the top of respective test tubes partially filled with water or water solution of 0.2% per volume with biorootz, in such a way that the rootlets could be initially grow moistened by the water risen due to capillarity within the tubes through a filter paper (see Fig. 2). The experiment was replicated 3 times and repeated for 2 more plant seeds (pea [*Pisum sativum*] and switch grass [*Panicum virgatum*]).



Fig. 3. The initial root development of cynara supplied with pure water [C] or water with biorootz in solution 0.2% volumetric [B].

It can be seen that the effect of the treatment was apparent throughout the first 8 days of initial development. However, this effect was more pronounced during the first two days. As a matter of fact the days that the treated seed reaches the stage of upper left quadrant is 2+6=8 days since sowing (experiment 1 initiation) whereas it seems that it will take at least one week more for the untreated seed to reach the same growing stage (combination of exps 1 and 2).

Even 8 days after germination, a substantial difference between the treated and the control seed is obvious in Fig. 3 (down-right quadrant).

The research is being continued; however based on the above experiments we conclude that the treatment of the seed with biorootz will result in a faster germination and a better initial grow of the plants, and due to its low cost is advisable for cynara cardunculus.

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Natural liquid supplement for broilers

NutroEgg

Natural liquid supplement for laying hens

specially developed supplement for broilers

specially developed supplement for laying hens

Our natural products : GMP+ FSA certified

Our natural products Nutro Meat (for broilers) and Nutro Egg (for laying hens) consist entirely of premium herbs and high-quality plant extracts and are GMP+ FSA certified.

Premium herbs and plants

The premium herbs and high-quality plant extracts in our products contribute to reducing and preventing broiler and layer health problems.

The active substances in herbs and plants have positive effects on the health, strength and performance of broilers and laying hens.

Ingredients

The most important ingredients in our products:

- Ascophyllum nodosum (seaweed)
- Foeniculum vulgare (fennel)
- Schisandra chinensis (pepperberry/wu wei zi)

Synergy of ingredients and special treatment

The mutual synergy of the ingredients in our products, the coordinated dosage and our special heating and fermentation treatment method, result in the combined effect that our products are more effective than the cumulative effect of all the ingredients together.

Proven impressive results (*see also the official reports below)

- enhanced feed conversion rates (FCR);
- strengthened immune system;
- improved liver function;
- improved disease resistance;
- supressed E.coli;
- less yolk residual inflammation;
- lower mortality;
- lower broiler rejection by the slaughterhouses;
- higher final body weight (e.g. +21% in India, +23% in Kenya, +26% in Indonesia);
- less/no antibiotics needed: AMR prevention and reduction;
- healthier and more sustainable chicken meat and eggs.

Our natural products are easy to use and provide a high return on investment. Our products are free from gluten, flavor enhancers and colorants.

SATISFIED USERS OF NUTRO MEAT®

'I see better FCR, health, vitality and weight.'
Mr. Jacobs-Migielsen - broiler entrepeneur

'Better growth and reduction of E.coli pressure.'
Mr. Dick Koldewé - broiler advisor

'Low mortality and high weight gain.'
Mr. Stephen Kinyanjui - broiler veterinarian

SCIENTIFIC RESEARCH on NUTRO MEAT® and its ingredients

NUTRO MEAT: POSITIVE EFFECT ON PRODUCTION PERFORMANCE

'Nutro Meat has a positive effect on production performance, carcass quality, blood profile, parasitology tests and small intestine morphology.'
Animal Husbandry Faculty of Islamic University of Malang, East Java, Indonesia - 2025

ASCOPHYLLUM NODOSUM (SEAWEED) : REDUCES E. COLI - STRENGTHENS IMMUNE SYSTEM - IMPROVES WEIGHT GAIN, FEED INTAKE AND FCR

'Seaweeds have antimicrobial properties; they played an essential role in reducing enterohemorrhagic E. coli, as well as in the growth of Salmonella spp.'
The Trafford Group of Colleges, Manchester, United Kingdom - 2022

'Nutritional supplementation of seaweed improved intestinal health, immune system, body weight, feed intake, feed conversion ratio and meat quality of broilers.'
Department of Animal Science and Aquaculture, Faculty of Agriculture, Dalhousie University, Truro, NS, B2N 5E3, Canada - 2022

'Ascophyllum nodosum phlorotannin exhibits bactericidal activity against Escherichia coli O157:H7 strains.'
ANSES, Laboratory of Ploufragan-Plouzané-Niort, Unit of Hygiene and Quality of Poultry and Pork Products, BP53, 22440 Ploufragan, France - 2022

FOENICULUM VULGARE (FENNEL) : IMPROVES PERFORMANCE - INCREASES ANTIBODY TITERS AGAINST INFECTIOUS DISEASES

'Fennel seeds have several biological effects in poultry, including improved performance, higher proliferation of immune cells, reduced oxidative stress and increased antibody titers against infectious diseases.'

'Fennel seed supplementation has multiple beneficial effects on poultry growth and health'
Department of DETO, Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', 70010 Bari, Italy - 2022

'Broilers feeds containing different levels of fennel seed showed improved weight gain and feed efficiency.'
'Dietary supplementation of fennel improved gut morphological development and thus promoted healthy and efficient development in Cobb broilers.'
College of Animal Science and Technology, Gansu Agricultural University, Lan Zhou, Gansu, China - 2021

SCHISANDRA CHINENSIS (PEPPERBERRY) : IMPROVES ANTIBODY TITERS AGAINST NEWCASTLE DISEASE - REGULATES BACTERIA

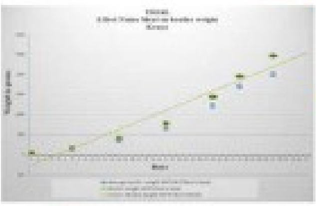
'Significantly improved antibody titers against Newcastle disease virus and lymphocyte proliferation in broilers.'

'In terms of microbial barriers, plant-derived polysaccharides regulated the abundance of beneficial bacteria and harmful bacteria, and could be regulated by the content of shortchain fatty acids. In terms of chemical and physical barriers, plant-derived polysaccharides promoted mucin, digestive enzymes and villus morphology, and reduced intestinal permeability.'

'In terms of the immune barrier, plant-derived polysaccharides upregulated immunoglobulins, cytokines and cellular mediators in the intestinal tract, and regulated them through the corresponding signaling pathways.'
College of Animal Science, Inner Mongolia Agricultural University, Hohhot 010018, China - 2022

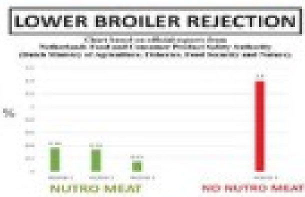
The above is just a small sample of the total number of positive research results on Nutro Meat and its ingredients. If you wish, we can send you the full research from the worldwide universities and research institutes. Ask us!

IMPRESSIVE RESULTS WORLDWIDE

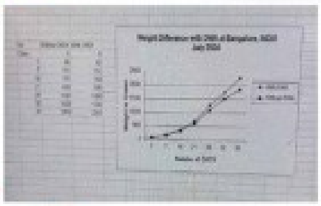


Kenya:
extra body weight gain
+ 23% with Nutro Meat

Indonesia:
extra body weight gain
+ 26% with Nutro Meat



The Netherlands:
less broiler rejection
by the slaughterhouses



India:
extra body weight gain
+ 21% with Nutro Meat

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NutroCorp
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We support professional poultry companies with our knowledge, our experience and with specially developed natural supplements to optimize the profitability of our clients and to increase sustainable, healthy and safe food production.
Our innovative natural feed supplements are GMP+ FSA (Feed Safety Assurance) assured and support broilers and laying hens to stay healthy and vital and to deliver healthy and nutritious chicken meat and eggs.
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Nutro Meat and Nutro Egg are developed and produced in The Netherlands and are recommended by veterinarians. Our safe, natural products ensure a better health, a stronger immune system and optimal resistance to infections and viruses.
Positive effects:
* FCR improves
* mortality decreases
* slaughterhouse rejection decreases
* less/no antibiotics needed
* better and more sustainable chicken meat/eggs
* total net margin increases by at least 10%
NutroMeat-ID
SDG's Certified For a Sustainable World
Feed Safety GMP+ Assurance
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Our number 1 mission is to support professional poultry companies in producing HEALTHY, SAFE and NUTRITIOUS food for everyone, worldwide.
Our number 2 mission is to apply our innovative natural supplements (GMP+ FSA assured) to achieve the Sustainable Development Goals of the United Nations.
Our number 3 mission is to make a substantial contribution to PREVENT and REDUCE Antimicrobial Resistance (AMR).
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PROJECT RESEARCH REPORT COLLABORATION
HERBS ADDITIVE FOR BROILER PRODUCTION

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Nutrocorp International
PT. SBDI Center Foundation Wise-use International Netherland
PT. Megatama Citra Dynamics (DMC)

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Jeroen Rijnenberg
SBDI Center Foundation Wise-use International Netherland
Theo
Nutrocorp International
Dessy
PT. Megatama Citra Dynamics (DMC)

2025

1

VALIDITY REPORT

PROJECT RESEARCH REPORT COLLABORATION

HERBS ADDITIVE FOR BROILER PRODUCTION

Malang, February 25th 2025
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NPP. 242507197932243

Universitas Islam Malang,
Animal Husbandry Faculty
Dean,

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NPP. 193.02.00019

SBDI Center Foundation International
Netherland,
CEO,

Jeroen Rijnenberg

SBDI CENTER
Sustainable Business Development and Innovation Center

Wise-use International BV
CEO,

Dorotheus Wisman

TABLE OF CONTENT

SUMMARY

Under natural conditions, animals have the instinct to seek out and consume herbs to treat illnesses. Some livestock such as cats, dogs, cats, and horses seek out specialized medicinal plants when they are sick. Traditional medicinal plants such as Ayuverdic, Indian, Chinese, Western, or African medicinal plants are generally a comprehensive therapy and utilize all parts of the plant including roots, seeds, or leaves that are thought to have more effective properties. Traditionally, herbs or parts of selected plants contain more than one active pharmacological component.

Modern herbal medicine is more pharmacological in nature, with scientists using specific active components, extracting and purifying them and utilizing the active compounds by isolating them. This treatment is not a holistic approach because one active component can produce different actions and have different effects. When all parts of the herb or parts of the plant (leaves, roots, seeds) are combined with other herbs, it will work synergistically and harmoniously because it produces a positive effect that runs naturally. But on the other hand, modern medicine continues to develop to identify and isolate the active components contained in herbal plants.

The output achieved by this research is to obtain complete and comprehensive data on the implementation of additive. The response of poultry production to the use of herbal plants and their derivatives as feed additives and feed supplements varies widely. The quality and quantity of active substances contained in herbal plants affect the response of poultry production. Several factors affect the effectiveness of phytobiotic feed additives, including; plant parts and properties owned, genetic variation of plants, age of plants, dosage of use, extraction method, harvest time, and its interaction with other compounds also explain the occurrence of different responses to production performance such as weight gain and feed conversion. In addition, the effectiveness of phytobiotics as feed additives can be influenced by internal and external factors such as livestock nutritional status, infection, feed composition, and the environment.

Commercial herbal additives with the brand name Nutromeat have a positive effect on production performance, carcass quality, blood profile, parasitology tests, small intestine morphology and are more economical for broiler maintenance.

Key words: Herbs, additives, broiler, production

CHAPTER I INTRODUCTION

1.1. Background

The broiler chicken farming industry continues to grow rapidly along with the increasing public demand for quality animal protein sources. The need for animal protein sources including meat and eggs tends to increase in terms of sales and demand, especially in Indonesia along with the increasing population (BPS, 2022). Meat type chickens or better known as broiler are currently in the top position as suppliers of meat needs for the community (Beski et al., 2015). The main challenge in broiler production is maintaining production performance, cage environment quality, and chicken health. The increasing research conducted throughout the world on the use of herbal plants in animal feed as feed additives aims to reduce the use of antibiotics as growth promoters in animal feed. Various efforts have been made to replace antibiotics with other products such as probiotics, prebiotics and also herbal plants to maintain livestock productivity. Herbal plants are natural growth promoters and are safe for human consumption because they leave no residue in livestock products. The less antibiotics are used in livestock farming, the less residue is found in these livestock products. The use of herbal plants is also cheaper so it can reduce feed costs.

(Langeroudi et al., 2008) conducted a test to compare the use of antibiotics, probiotics, and two herbal plant preparations. The results of the study showed that herbal plant preparations can effectively replace virginiamycin as a growth promoter in broiler chickens. (Basmacioğlu et al., 2020) showed that replacing antibiotics with essential oils is effective in supporting growth in broiler chickens. The conclusion obtained is that antibiotics can be replaced with herbal plants and essential oils without having a negative effect on growth.

The use of antibiotic compounds has decreased and even in some countries have banned the use of antibiotics as additives in animal feed, this is due to the presence of antibiotic residues that can be harmful to consumers of livestock products, in addition antibiotics can create resistant microorganisms in the human body or livestock, especially pathogenic bacteria including *Salmonella sp.* One of the safe alternatives used as feed additives in rations and drinking water is a concoction of herbal plants that are relatively cheaper and easier to obtain, so that it will provide benefits for farmers. In addition, herbal concoctions are also able to lower cholesterol levels in the body of livestock so that it will affect livestock products including eggs and meat.

Herbal have long been known by Indonesian people as medicine and to improve metabolism in the body. Popular scientific reports show that the use of various herbal ingredients for humans is also effective in suppressing various diseases in livestock, but scientific facts have not revealed much. Improving metabolism through the administration of herbal concoctions will indirectly improve livestock performance through the bioactive substances they contain. Thus, livestock will be healthier because they have better immunity, and according to observations by farmers, the aroma of meat and eggs from chickens given herbal medicine is not fishy compared to chickens not given herbal medicine (Agustina, 2006).

According to (Agustina, 2014), the high price of commercial medicines and feeds and the increasing public awareness of the importance of food safety that they consume encourage the idea of utilizing various traditional plants both as feed supplements and/or medicines. Indonesia is very rich in traditional plants that have positive functions and have not been optimally explored until now. The use of antibiotics as feed additives in rations has had negative impacts or influences, including bacterial residues and resistance. In addition, in Indonesia the use of antibiotics in livestock is uncontrolled, resulting in negative impacts on livestock and humans who consume livestock products. Currently, safe and natural alternative ingredients are needed to replace the function of antibiotics, including herbs. The use of herbs as feed additives in broiler rations aims to replace the use of antibiotics as growth promoters and disease prevention in poultry so that livestock and humans can avoid antibiotic residues and bacterial resistance. The benefits of using herbs in poultry rations are as feed additives that have a positive impact on increasing livestock growth and health. In addition, the use of herbs is relatively cheaper than antibiotics, so the use of herbs must now be increased and in the future in a modern way.

Based on this background, the researcher is interested in conducting a study by evaluating Herbs Additives on production performance, enclosure microclimate, product quality, feed efficiency, blood profile, and parasitology test, morphology of the intestine digestive tract in broiler Enclosure. This study was conducted to support the development of a more environmentally friendly and sustainable poultry system.

1.2. Formulation of the problem

How does the use of herbal additives affect the maintenance in closed cages on aspects of production performance, enclosure *microclimate*, product quality, feed efficiency, blood profile, and morphology of the broiler digestive tract.

1.3. Research purposes

This study aims to provide comprehensive information on the use of herbal additives in broiler enclosure rearing on aspects of production, microclimate, product quality, feed efficiency, blood profile, and morphology of the digestive tract of broilers.

1.4. Benefits of research

This study provides information to all parties who need information about the use of herbal additives in enclosure broiler rearing on aspects of production, microclimate, product quality, feed efficiency, blood profile, and morphology of the broiler digestive tract.

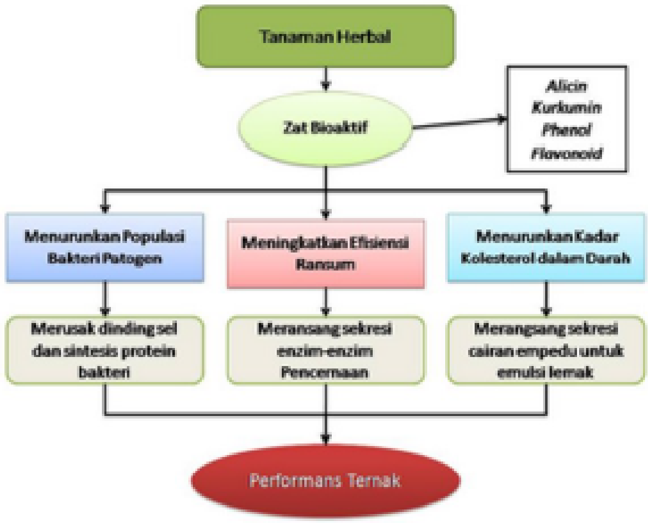
CHAPTER II LITERATUR REVIEW

2.1. State of The Art

Phytogenic feed additives are derivatives of plant products used in animal feed to improve the performance of livestock production. Phytobiotics have begun to be widely developed over the past few years, especially for poultry and pigs. The movement to use phytobiotics has grown since European countries opposed the use of antibiotics because they cause resistant effects on pathogenic microorganisms. Phytobiotics can be used as growth promoters such as organic acids and probiotics that can be popularized in livestock nutrition.

Phytogenic is a new class in the feed additive group so that information related to the mechanism of action, action, and technical application is still very limited. In addition, what makes the use of phytobiotics complex is its diversity caused by several factors, namely plant type, processing, and composition. Several studies have examined the mixture of several active components in phytobiotics and how they affect livestock production performance. Unfortunately, there are still few studies on their effects on livestock physiology.

The mechanism of action of phytobiotics on poultry physiology is summarized in the following illustration.



Each country or region has local herbal plants that are germplasm that are worthy of being developed as "exotic" plants, although currently Chinese and Ayurvedic herbal plants are becoming popular in various parts of the world today. Herbal plants from Western countries are classified based on their pharmacological activity. Medicinal plants have amazing active substance content, for example willow bark (contains salicylate which is similar to aspirin and functions as an effective pain reliever). Of course, with a lower dose when compared to aspirin. Digitalis or foxgloves, is known as an effective heart medicine because it has an action to maintain all heart functions. Dandelion (as a medicine for diabetes and improves blood circulation), Periwinkle or Vinca, has the potential to be a medicine for cancer).

Poultry production responses to the use of herbal plants and their derivatives as feed additives and feed supplements vary widely. (Cross et al., 2007) concluded that the quality and quantity of active substances contained in herbal plants affect the production response of poultry. Several factors affect the effectiveness of phytobiotic feed additives, including; plant parts and properties owned, genetic variation of plants, age of plants, dosage of use, extraction method, harvest time, and its interaction with other compounds also explain the occurrence of different responses to production performance such as weight gain and feed conversion. In addition, the effectiveness of phytobiotics as feed additives can be influenced by internal and external factors such as livestock nutritional status, infection, feed composition, and the environment (Puvača et al., 2022)

2.2. Roadmap Study

Roadmap study in accordance with Research Plan of Universitas Malang 2020 - 2025 was Food

Security and Independence is the topic of increasing agricultural production through improvements. management and technology with the output resulting in innovations to improve agricultural products in meaning wide.

Animal Science Faculty, Universitas Islam Malang have 5 major concentrations:

1. Animal Production,
2. Animal Nutrition and Feeding,
3. Technology of Animal Product,
4. Animal Breeding and Reproduction, and
5. Animal Husbandry Sosio-Economic

Human resources of Animal Science Faculty were 17 persons with different expertise, i.e.

No.	Lecture	Field	Expertise
1	Ir. M. Farid Wadjdi, M.P.	Animal Nutrition and Feeding	Animal Nutrition and Feeding
2	Dr. Ir. Usman Ali, M.P.		Nutrition and Feed Technology
3	Prof. Dr. Ir. Badat Muwakhid, M.P., IPM.		Animal Nutrition and Feeding
4	Dr. Ir. Umi Kalsum, M.P.		Animal Nutrition and Feeding
5	Ir. Brahmadhita Pratama Mahardika, S.Pt.,M.Si.,IPP.		Nutrition and Feed Technology
6	Dr. Ir. Sumartono, M.P.	Animal Production	Beef Cattle Production
7	Ir. Dedi Suryanto, M.P.		Anatomy and Physiology
8	Dr. Ir. Inggit Kentjonowaty,M.P.		Dairy Livestock Production
9	Dr. Dyah Lestari Yulianti, S.Pt.,M.P.		Poultry Production
10	Prof. Dr. Ir. Mudawamah, M.Si., IPM., ASEAN Eng.	Animal Breeding and Genetic	Genetic and Breeding
11	Dr. drh. Nurul Humaidah, M.Kes.		Animal Reproduction
12	Nisa'us Sholikah, S.Pt., M.Pt.		Animal Reproduction
13	M. Mas'ud Chabiburrochman, S.Pt., M.Sc.		Dairy Livestock Reproduction
14	Ir. Irawati Dinasari,M.P.	Technology of Animal Product	Technology of Animal Product
15	Oktavia Rahayu Puspitarini, S.Pt. M.Si.		Technology of Animal Product
16	Ir. Sri Susilowati,M.M.	Animal Husbandry Sosio-Economic	Animal Husbandry Sosio-Economic
17	Dr. Ir. Dewi Masyithoh, S.P., M.Pt		Animal Husbandry Sosio-Economic

CHAPTER III MATERIAL AND METHODS

The research was conducted in December-January 2024. Located at PT Dinamika Megatama Citra, Boro Dsn., Tawangarjo Village, Karangploso District, Malang Regency, East Java.

3.1. Research Materials

The materials used in this research are:

1. DOC of Cobb strain broiler chickens: 3,400 in control cages and 3,200 in trial enclosure, totaling 6,600 in total.
2. Chicken feed with commercial code SB 20 (0-7 days), SB 21 CR (8-14 days), SB 21 P (15-30 days), SB 22 P (31 days-harvest)
3. Commercial herbal additive products with the trademark nutromeat have the following main ingredients: Seaweed (*Ascophyllum nodosum*), Fennel (*Foeniculum vulgare mili*), and five-flavored berries (*Schisandra chinensis*).
4. Drinking water comes from a drilled well which flows into the house automatically.
5. The equipment used in this study were 2 postal chicken cages, baby chick feeders, pipes, nipple drinkers, brooders, water tanks, chicken scales, NH_3 test product, CO_2 sensors.

3.2. Method

The study used a two-treatment experimental method, P0=0 ml herbal additive/1000 liters of drinking water (control) and P1 = 5 ml herbal additive/1000 liters of drinking water.

3.3. Research Procedures

The research preparation carried out was to prepare 2 postal cages, food and drink places and other equipment, prepare drinking water with the addition of herbal additives. The treatment of giving *herbal additives* through drinking water was carried out for 40 days, given to chickens from the age of 0 days, the number of chickens in this treatment was 6600 divided into 2 treatments and 20 replications, a total of 2 cages and each cage contained 3,400 (P0) and 3,200 (P1). The procedure for data collection and analysis of research data is presented in Table 2.

Table 2. Procedures for data collection and analysis of research data

Research Variables	Data Collection Procedure	Data analysis
Production appearance		
Feed consumption (g/birds/day)	Every day, cumulative feed consumption	Descriptive
Body Weight (g/birds)	Weighed every week 50 animals per treatment aged 7 days, 14 days, 21 days, 28 days, 35 days and 40 days.	Statistics compare means independent sample t using SPSS software
Body Weight Gain (BWG)	$\mathit{Final\ Body\ Weight} - \mathit{Initial\ Body\ Weight}$	Statistics compare means independent sample t using SPSS software
Feed Conversion	$= \frac{\mathit{Feed\ Consumption}}{\mathit{Body\ Weight\ Gain}}$	Statistics compare means independent sample t using SPSS software
Microclimate		
Ammonia (NH_3) and CO_2	Data collection at the age of 15 days, 22 days, 30 days, and 36 days. Conducted at 9 points of the cage. These points are divided based on three main zones in the cage, namely the front, middle, and back. Each zone is then divided into three sub-measurement points, namely right, left, and middle. Data collection is carried out at 10.00 WIB every 1 week using the NH3 prodac test tool, CO_2 sensor	General linear model statistics (two factors, *herbs additive treatment* and observation) using SPSS software
Carcass characteristics		
Carcass percentage (%) Carcass cut percentage (%) Abdominal fat (g/bird)	Each treatment took 10 samples and weighed them.	Statistics compare means independent sample t using SPSS software
Blood Constituent	For physiological status parameters, namely complete blood (hemoglobin, hematocrit, erythrocytes, leukocytes), glucose levels. Obtained by taking blood from the brachial vein. For complete blood samples, 1cc was taken and then analyzed with a blood analyzer. Chicken samples used for blood analysis were 6 (3 for each treatment). Examination method: Serological examination is intended to determine the levels in the sample. Examination using *ELISA READER* with the *Diatek DR-200BC brand*	Statistics compare means independent sample t using SPSS software

<table>
<tr><td>Small Intestinal Morphology</td><td>3 samples of the jejunum, ileum, and duodenum were taken from each treatment and then continued with histological testing.</td><td>Statistics compare means independent sample t using SPSS software</td></tr>
<tr><td>Parasitology Test</td><td>Stool sample information for severity of infection by oocyst count per gram (OPG count) Suspect E. acervulina and E. Tenella
Result Interpretation:
<table>
<tr><td>Severity Infection</td><td>OPG count</td></tr>
<tr><td>Mild infection</td><td><10,000</td></tr>
<tr><td>Moderate Infection</td><td>10,000-15,000</td></tr>
<tr><td>Severe Infection</td><td>>15,000</td></tr>
<tr><td colspan="2">(Lunden et al., 2000; Matthew et al., 2022)</td></tr>
</table></td><td>Descriptive</td></tr>
</table>

CHAPTER IV RESULTS AND DISCUSSION

4.1. Results

4.1.1. Production Performance

Table 1. Effect of herbs additive on broiler production performance

Parameters	Age (days)	Control	Herbs Additive
FI (g/bird/day)	7	29.6	31.5
	14	60.0	63.5
	21	92.6	81.6
	28	93.6	83.1
	35	126.7	100.9
	40	111.9	101.8
BW (g/bird)	7	170.0	165.0
	14	486.0	480.0
	21	950.0	950.0
	28	1480.0	1470.0
	35	1920.0	1945.0
	40	2340.0	2360.0
Daily Gain (g/bird)	7	19.0	22.0
	14	76.0	75.0
	21	73.0	86.0
	28	100.0	89.0
	35	60.0	85.0
	40	81.0	92.0
Mortality cum. (%)	7	0.71	0.69
	14	1.88	1.63
	21	4.68	4.31
	28	5.71	5.97
	35	7.15	7.06
	40	8.00	7.88
FCR	7	1.22	1.33
	14	1.05	1.13
	21	1.06	1.10
	28	1.11	1.15
	35	1.25	1.23
	40	1.26	1.24

mean
FI=Feed Intake, BW=Body Weight, DG=Daily Gain, FCR=Feed Conversion Ratio

4.1.2. Microclimate Parameters (*Closed House*)

Table 2. Effect of herbs additive on microclimate parameters (*closed house*)

Parameters	Observation	Control	Herbs Additive
Temperature (ºC)	Day 15	29.47±0.21	31.73±0.26
	Day 22	28.51±0.36	29.02±0.44
	Day 30	28.26±0.36	27.68±0.34
	Total	28.66±0.58	29.06±1.69
RH (%)	Day 15	81.14±3.70	81.21±2.43
	Day 22	80.46±2.58	82.12±1.80
	Day 30	76.33±1.55	72.87±1.91
	Day 36	74.18±1.21	76.35±1.09
	Total	78.09±3.71	78.04±4.29
Wind speed (m/s)	Day 15	0.00±0.00	0.00±0.00
	Day 22	0.64±0.22	0.50±0.14
	Day 30	1.36±0.22	1.33±0.33
	Day 36	1.36±0.18	1.40±0.44
	Total	0.84±0.60	0.81±0.65
NH_3 (ppm)	Day 15	3.74±0.72	3.77±0.75
	Day 22	3.58±0.42	3.14±0.75
	Day 30	4.61±0.70	4.26±0.62
	Day 36	3.99±0.40	4.51±1.67
	Total	4.00±0.70	3.91±1.09
CO_2 (ppm)	Day 15	663.44±47.25	637.00±96.82
	Day 22	505.89±13.29	503.56±36.99
	Day 30	516.40±39.85	509.70±24.01
	Day 36	579.63±54.85	588.88±58.37
	Total	564.58±75.33	557.58±80.85

Analysis of Variance	T (ºC)	RH (%)	WS (m/s)	NH_3 (ppm)	CO_2 (ppm)
	Sig.				
Treatment	0.000	0.830	0.539	0.762	0.592
Observation	0.000	0.000	0.000	0.001	0.000
Treatment*Observation	0.000	0.001	0.692	0.314	0.783

mean±standard deviation
$p<0.05$
RH=Relative Humidity, T=Temperature, WS=Wind speed

4.1.3. Carcass Characteristic

Table 3. Effect of herbs additive on broiler carcass characteristics

Parameters	Herbs Additive	Mean	SEM	*Sig.*
Final Body Weight (kg/bird)	Control	2157.00±282.41	89.31	.000
	Herbs Additive	2720.00±214.99	67.99	
Carcass Percentage (%)	Control	68.43±5.28	1.67	.968
	Herbs Additive	68.54±6.66	2.11	
Abdominal Fat (g/bird)	Control	21.10±9.54	3.02	.243
	Herbs Additive	15.50±11.14	3.52	
Carcass cut-up parts (% live weight)				
Thigh (%)	Control	9.80±2.20	0.70	.043
	Herbs Additive	9.90±1.04	0.33	
Wings (%)	Control	7.64±1.56	0.49	.896
	Herbs Additive	7.16±0.75	0.24	
Breast (%)	Control	31.70±3.93	1.24	.089
	Herbs Additive	34.98±4.21	1.33	
Drum Sticks (%)	Control	9.18±0.76	0.24	.660
	Herbs Additive	9.33±0.73	0.23	
Back (%)	Control	9.04±0.77	0.24	.0760
	Herbs Additive	8.92±1.03	0.33	

Mean ± standard deviation
P<0.05
SEM=Standard Error Means

4.1.4. Blood Constituents

Table 4. Effect of herbs additive on broiler blood constituents

Parameters	Treatment	Mean	SEM	*Sig.*
Super Oxide Dismutase (U/mL)	Control	31.40±0.86	0.50	0.040
	Herbs Additive	36.52±2.82	1.63	
Malondialdehyde (nmol/mL)	Control	11.19±1.08	0.62	0.094
	Herbs Additive	8.99±1.37	0.79	
Cholesterol (mg/dL)	Control	192.94±39.69	22.92	0.324
	Herbs Additive	152.15±48.74	28.14	
LDL (mg/dL)	Control	68.87±3.04	1.76	0.009
	Herbs Additive	34.55±11.98	6.92	
HDL (mg/dL)	Control	67.55±2.32	1.34	0.000
	Herbs Additive	25.06±4.13	2.38	
Triglycerides (mg/dL)	Control	97.31±0.62	0.36	0.044
	Herbs Additive	111.47±8.41	4.86	

Mean ± standard deviation
P<0.05
SE=Standard Error Mean, LDL=Low Density Lipoprotein, HDL=High Density Lipoprotein

4.1.5. Small Intestinal Morphology

Table 5. Effect of herbal additives on broiler small intestinal morphology

Treatment	Intestine	Villus Height (micro-meter)	Crypt Depth (micro-meter)	VH:CD
Control	Duodenum	1005.54±35.79	159.44±24.46	6.38±0.77
	Jejunum	1004.15±212.59	204.65±13.86	4.88±0.75
	Ileum	1076.82±195.10	298.33±94.49	3.72±0.69
	Total	1028.84±149.77	220.81±78.70	5.00±1.32
Herbs Additive	Duodenum	1193.14±119.87	231.99±41.63	5.20±0.57
	Jejunum	1150.43±155.60	232.90±12.24	4.94±0.60
	Ileum	1127.94±152.32	203.47±21.31	5.60±1.07
	Total	1157.17±127.54	222.78±28.18	5.25±0.74
Analysis of Variance		*Sig.*		
Treatment		0.107	0.927	0.496
Intestine		0.955	0.143	0.057
Treatment*Intestine		0.745	0.019	0.014

Figure 1. Illustration of jejunum histology. Left (control), right (herbs additive treatment)

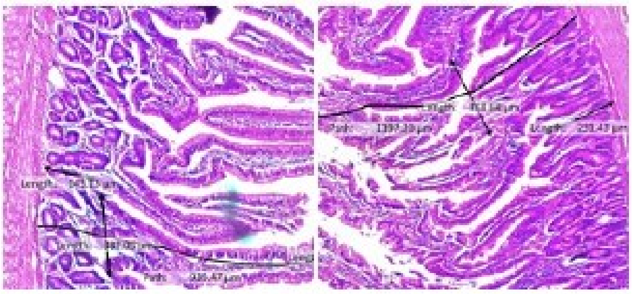


Figure 2. Illustration of duodenal histology. Left (control), right (herbs additive treatment)

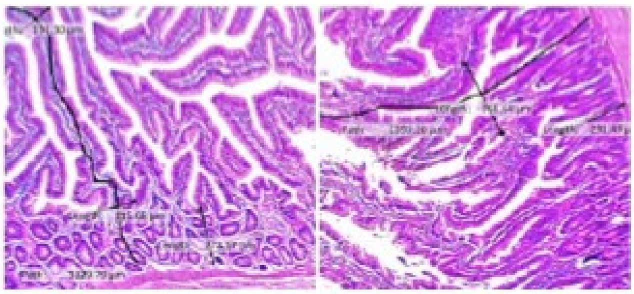


Figure 3. Illustration of ileum histology. Left (*control*), right (herbs additive treatment)

4.1.6. Parasitology Test

Table 6. Average number of *E. acervulina* / *E. tenella oocytes* in broiler excreta samples (oocyst/gram)

Treatment	Age (days)	Oocyst/gram	Remark
Control	5	0.0	Mild infection
	15	0.0	Mild infection
	22	100.0	Mild infection
	30	31,000.0	Severe infection
	36	2,250.0	Mild infection
Herbs Additive	5	0.0	Mild infection
	15	0.0	Mild infection
	22	5,759.0	Mild infection
	30	4,750.0	Mild infection
	36	850.0	Mild infection

Note: Parasitology test results PT. Provela (Professional Veterinary Laboratory)

4.1.7. Production Efficiency

Table 6. Comparison of production costs using herbs additive (Nutromeat)

Production Cost		Unit	IDR/Unit	Control		Herbs Additive	
				Amount	Total Cost (IDR)	Amount	Total Cost (IDR)
DOC		box	600,000	34	20,400,000	32	19,200,000
Feed	Pre-starter	sack	422,500	21	8,872,500	21	8,872,500
	Starter	sack	417,500	50	20,875,000	49	20,457,500
	Finisher	sack	411,000	114	46,854,000	102	41,922,000
Supportive	Gumbonal	100 g	15,000	15	225,000		
	Kumavit	250 g	34,000	12	408,000		
	Chikovit	liter	111,000	1	111,000		
	Vitamin Eco	250 g	23,000	4	92,000		
	Probiotic	kg	40,000	2	80,000		
	Herbs additive	liter	200,000			23	4,600,000
Vaccine		vial	45,000	5	225,000	6	270,000
			TOTAL PRODUCTION COST		98,142,500		95,322,000
			TOTAL COST OVK/KG		234		1,029
			TOTAL COST OF FEED/KG		15,708		15,053
			TOTAL COST DOC/KG		4,183		4,056
			TOTAL COST/KG		20,125		20,138

Income Production	Unit	Control	Herbs Additive
Initial Population	birds	3,400	3,200
Final Population	birds	3,118	2,940
Depletion	%	8.29	8.13
Average Final BW	kg/bird	1,564	1,610
Harvest Tonnage	kg	4,877	4,733
Price/Kg BW	IDR/kg BW	20,000	20,000
INCOME PRODUCTION		97,531,040	94,668,000

4.2. Discussion

4.2.1. Production Performance

Based on the statistical analysis of the study presented in Table 1., the addition of herbal additives provided higher broiler production performance in each when compared to the control. Production performance parameters include: feed consumption, body weight, body weight gain, mortality percentage, and feed conversion. In general, the mechanism of action of active ingredients of medicinal plants in the animal's body plays a role in influencing the nervous system, digestive conditions, and immunity. Sensory factors are factors that greatly influence feed consumption. Consumption of medicinal plants can stimulate the central nervous system, then stimulate the salivary glands and secretion of digestive fluids from the stomach, liver, pancreas, and small intestine which are useful for controlling the appropriate pH for the effectiveness of digestive enzymes (Ulfah, 2006).

The active ingredients of herbal additives are thought to have a similar effect on the broiler digestive system. (Widodo, 2010) stated that the hydrogen potential (pH) in the poultry digestive tract increases from the anterior to the posterior. The effectiveness of the digestive process is greatly influenced by the pH conditions of each part, meaning that the enzymes and products secreted in the digestive tract have such characteristics and will have sensitivity to pH, temperature, transit time, population, type of microbes, and digesta viscosity (Widodo, 2010).

One of the very important factors required for animal health is the balance of microflora composition in the poultry digestive tract. More than 90% of the total bacteria should be beneficial bacteria such as Lactobacilli and Bifidobacteria, and only a very small proportion of harmful bacteria (pathogens) such as *Escherichia coli* and *Staphylococci* (Ulfah, 2006).

Medicinal plants and spices are known to have antimicrobial effects in vitro against pathogenic bacteria including fungi (Adiguzel et al., 2009; Banaszak et al., 2021; Burt, 2004; Dorman & Deans, 2000; Hammer et al., 1999; Smith-Palmer et al., 1998). The mechanism of action of phytobiotic feed additives reported by (Jamroz et al., 2003), namely stimulating mucus secretion in the digestive tract of broiler chickens whose effect is to weaken the adhesion of pathogenic microorganisms and this contributes to stabilizing the balance of the microorganism population in the animal's digestive tract (*eubiosis*). These observations support the hypothesis that phytobiotic feed additives provide beneficial effects on the presence of fungi in the digestive tract

4.2.2. Microclimate Parameters

Based on the statistical analysis in Table 2, it shows that herbal additives have an effect ($P<0.05$) on the cage microclimate, namely temperature, but have no effect ($P>0.05$) on humidity, wind speed, ammonia gas concentration and carbon dioxide, although numerically the herbal additive treatment produces lower microclimate parameters when compared to the control. This is thought to be because the closed house cage has a microclimate regulation system that always makes adjustments to create comfortable conditions for poultry. To anticipate unstable climate factors, currently in Indonesia many closed cages are used.

Closed house system is a type of cage that has good air ventilation settings with the help of an automatic control panel. The closed house system has ventilation that uses pressure generated by the fan (*exhaust fan*) and cooling pad as its cooling system. Closed house have zones in them, namely the zone close to the cooling pad/inlet, the middle zone and the zone close to the exhaust fan/outlet. The distribution of air flow, temperature, and humidity in the cage will differ from the inlet to the outlet (exhaust fan). Differences in the cage environment including temperature, humidity, and air speed result in differences in the effective temperature felt by broilers at the inlet to the outlet. Temperature is the main environmental factor that can affect broiler behavior, health and productivity. Different temperatures will affect production activity and the quality of eggs produced. Decreased feed intake is the starting point for other adverse effects such as decreased body weight, feed efficiency, egg production and egg quality. Decreased feed intake also causes decreased feed digestibility, decreased plasma protein and calcium levels.

4.2.3. Carcass Characteristic

Based on the results of statistical analysis, herbal additives gave a very significant effect on final body weight ($P<0.01$), but did not give any effect ($P>0.05$) on the percentage of carcass cuts and abdominal fat weight. This is suspected because the treatment in the control was also added with probiotics, which is thought to have a positive impact on carcass characteristics.

Research conducted by (Dhar & Dhar, 2019) to determine the effect of the use of herbal plants and growth promoters (Superliv and Xlivpro) to strengthen the research variables of production performance and carcass quality. The results of the study showed that there was an increase in growth in the treated group. In addition, there was also an increase in vitality, carcass production, and carcass quality. Poly herbal formulation to strengthen liver function increases the utilization of nutrients.

4.2.4. Blood Constituents

Based on the results of the statistical analysis presented in Table 4, herbal additives have a significant effect ($P<0.05$) on the blood chemistry profile of broilers, including: Super Oxide Dismutase, Malondialdehyde, Cholesterol, LDL, HDL.

In addition to reducing the population of pathogenic bacteria in the digestive tract, herbs are also able to reduce cholesterol in the blood of poultry so that poultry have low cholesterol content and smooth blood circulation (Lelono and Surya, 2023). The working mechanism is by stimulating the secretion of bile fluid which can emulsify fat. Bioactive substances contained in herbs are also able to stimulate the pancreas to secrete pancreatic juice containing digestive enzymes such as amylase, lipase and protease enzymes (Winarto, W.P., 2004; Yuliningtyas et al., 2019). Widodo (2002), stated that the substances contained can improve the work of the hormonal system, especially carbohydrate metabolism and metabolize fat in the body.

Most of the lipids in poultry feed are in the form of fatty acids, triglycerides, and cholesterol which will be absorbed in the small intestine to be degraded into fatty acids and glycerol which are then broken down into cyclomicrons. Cyclomicrons together with proteins (lipoproteins) are then absorbed into the blood circulation and transformed into VDL, HDL, LDL, and cholesterol. A small amount of lipids is converted into free fatty acids. The manifestation of lipid metabolism is lipids and cholesterol contained in meat products. Antioxidant compounds in various phytobiotics play a role in protecting feed lipids from damage caused by oxidation reactions. Research using herbal plants containing phenolic compounds has been shown to increase the stability of oxidation reactions of poultry derivative products such as chicken meat (Botsoglou et al., 2004)

4.2.5. Small Intestinal Morphology

Based on the results of statistical analysis presented in Table 5, herbal additives have a significant effect ($P<0.05$) on the morphology of the broiler small intestine including: villus height, and crypt depth. Phytobiotic feed additives affect the ecosystem of the digestive tract microbiota through the mechanism of controlling pathogenic microbes. The increase in digestive capacity in the small intestine is also an indirect effect of the eubiosis condition of the microflora that supports the stability of the poultry digestive tract. The presence of phytogenic will support livestock to obtain optimal immunity in critical conditions and increase the absorption of essential nutrients so that it will help livestock to grow better according to their genetic potential (Hashemi and Davoodi, 2010)

Medicinal plants are believed to be able to be used as multi-functional natural feed additives. Among the properties of medicinal plants are improving the condition of the digestive tract (pH balance and microflora), feed conversion, and increasing the digestibility of food substances (Ulfah, 2006).

4.2.6. Parasitology Test

Based on the results of parasitology tests (Table 6.) the use of herbal additives (Nutromeat) showed moderate infection when compared to without giving which identified high infection in observations of 30-day-old broilers. A number of herbal plants used for food can also be used to kill parasites in the digestive tract, for example ginger and essential oils that can kill worms. A number of studies have shown that ginger is more effective than *piperazine citrate*.

4.2.7. Production Efficiency

Based on the calculation of production costs (Table 6.), the use of herbal additives is more efficient when compared to without giving herbal additives, the average harvest weight is 1,610 kg (herbs additive) and 1,564 kg (control). The percentage of depletion and costs required to produce 1 kg of broiler body weight in the herbal additive treatment are lower than the control.

Conclusion

The response of poultry production to the use of herbal plants and their derivatives as feed additives and feed supplements varies widely. The quality and quantity of active substances contained in herbal plants affect the response of poultry production. Several factors affect the effectiveness of phytobiotic feed additives, including; plant parts and properties owned, genetic variation of plants, age of plants, dosage of use, extraction method, harvest time, and its interaction with other compounds also explain the occurrence of different responses to production performance such as weight gain and feed conversion. In addition, the effectiveness of phytobiotics as feed additives can be influenced by internal and external factors such as livestock nutritional status, infection, feed composition, and the environment.

Commercial herbal additives with the brand name Nutromeat have a positive effect on production performance, carcass quality, blood profile, parasitology tests, small intestine morphology and are more economical for broiler maintenance.

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Kepada
Dyah Lestari Yulianti
dyah_ly@unisma.ac.id

Tanggal Sampel
Selasa, 21 Januari 2025

Jenis Dokumen
Hasil Pemeriksaan

Jenis Pemeriksaan

	Histopatologi
	Biomolekuler
	Patologi Klinik
X	ELISA

Pemeriksa Sampel :
drh. Dewi Mariyam

Intansi : FAPET UNISMA

Jenis Sampel : Darah (*Serum)* dan Gross (*Usus*)

Metode Pemeriksaan : Pemeriksaan serologi ini dimaksudkan untuk mengetahui kadar pada sampel yang dikirimkan. pemeriksaan menggunakan ***ELISA READER*** dengan merk ***Diatek DR-200BC.***

Malang, 21 Januari 2025
Dokter Hewan Pemeriksa

SATWA SEHAT INDONESIA

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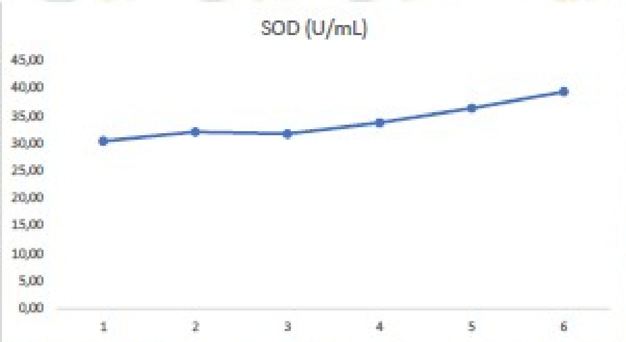
I. Pengukuran Kadar SOD

KODE SAMPEL	NILAI SOD (U/mL) ULANGAN KE-		RERATA	DEVIASI
	1	2		
P0U5	29,67	31,20	30,44	1,08
P0U7	30,99	33,21	32,10	1,57
P0U8	31,86	31,65	31,76	0,15
P1U1	33,21	34,29	33,75	0,76
P1U5	36,96	35,85	36,41	0,78
P1U9	40,95	37,83	39,39	2,21



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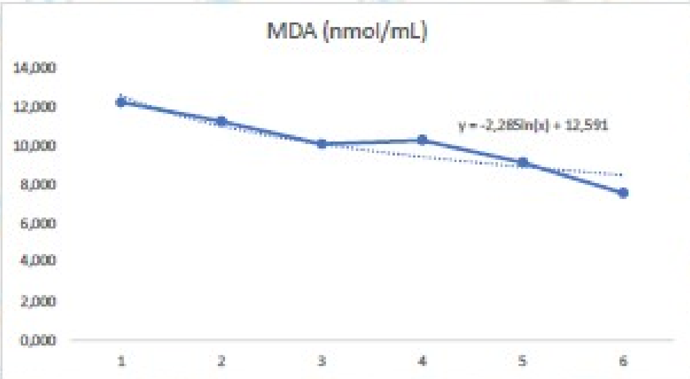
II. Pengukuran Kadar MDA

KODE SAMPEL	NILAI MDA (nmol/mL) ULANGAN KE		RERATA	DEVIASI
	1	2		
P0U5	12,019	12,446	12,233	0,302
P0U7	11,179	11,305	11,242	0,089
P0U8	10,045	10,115	10,080	0,049
P1U1	10,045	10,500	10,273	0,322
P1U5	9,058	9,205	9,132	0,104
P1U9	7,735	7,371	7,553	0,257



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III. Pengukuran Kimia Darah

KODE SAMPEL	NORMAL RANGE	NILAI CHOL ULANGAN KE		RERATA	SD
		1	2		
P0U5	150-250 (mg/dL)	150,59	151,46	151,03	0,62
P1U9		103,91	102,91	103,41	0,71
P1U1		200,02	201,76	200,89	1,23
P0U7		198,00	197,65	197,83	0,25
P0U8		230,50	229,41	229,96	0,77
P1U5		320,27	319,58	319,93	0,49

KODE SAMPEL	NORMAL RANGE	NILAI LDL ULANGAN KE		RERATA	SD
		1	2		
P0U5	20-60 (mg/dL)	10,26	11,25	10,75	0,70
P1U9		21,00	20,46	20,73	0,38
P1U1		41,24	42,63	41,94	0,98
P0U7		66,23	65,42	65,83	0,57
P0U8		72,21	71,61	71,91	0,42
P1U5		40,43	41,53	40,98	0,78

KODE SAMPEL	NORMAL RANGE	NILAI HDL ULANGAN KE		RERATA	SD
		1	2		
P0U5	15-30 (mg/dL)	69,28	70,46	69,87	0,83
P1U9		21,04	20,73	20,89	0,22
P1U1		64,77	65,68	65,23	0,64
P0U7		24,82	25,48	25,15	0,47
P0U8		28,46	29,83	29,15	0,97
P1U5		32,27	31,24	31,76	0,73

KODE SAMPEL	NORMAL RANGE	NILAI TG ULANGAN KE		RERATA	SD
		1	2		
P0U5	50-120 (mg/dL)	259,08	260,43	259,76	0,95
P1U9		216,25	217,14	216,70	0,63
P1U1		120,10	119,65	119,88	0,32
P0U7		96,14	97,24	96,69	0,78
P0U8		97,32	98,54	97,93	0,86
P1U5		103,47	102,65	103,06	0,58

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IV. Pengukuran Gambaran Usus

A. DUODENUM

KODE SAMPEL	TINGGI VILI						LEBAR APIKAL					
	LP1	LP2	LP3	LP4	LP5	RERATA	LP1	LP2	LP3	LP4	LP5	RERATA
P0U5	1329,79	1096,36	1093,63	1076,86	1007,65	**1120,86**	151,3	99,04	103,33	102,07	114,74	**114,10**
P0U7	1254,57	1253,62	1185,68	1241,78	1295,03	**1246,14**	76,47	84,11	84,93	77,18	147,36	**94,01**
P0U8	899,27	831,45	869,02	852,24	865,36	**863,47**	72,74	127,08	80,21	46,94	85,17	**82,43**
P1U1	931,27	962,06	1052,44	1105,86	973,98	**1005,12**	225,81	57,28	76,1	149,37	89,53	**119,62**
P1U5	933,26	918,89	953,16	1042,69	1553,66	**1080,33**	163,32	159,99	142,2	120,68	149,84	**147,21**
P1U9	1530,35	1366,25	1344,41	1089,28	1161,62	**1298,38**	106,46	66,85	114,6	130,24	148,02	**113,23**

KODE SAMPEL	LEBAR BASAL						LUAS VILI					
	LP1	LP2	LP3	LP4	LP5	RERATA	LP1	LP2	LP3	LP4	LP5	RERATA
P0U5	115,68	281,81	142,09	134,03	187,88	**172,30**	2346,51	4215,96	2597,49	2490,90	2657,62	**2861,20**
P0U7	155,58	101,48	93,56	147,64	106,09	**120,87**	3807,02	2766,13	2491,84	3617,22	2227,37	**2981,[illegible]**
P0U8	128,93	95,28	125,81	72,64	99,72	**104,48**	2493,21	1454,84	2232,08	2171,09	1878,55	**2045,05**
P1U1	302,48	263,02	133,74	92,09	176,78	**193,62**	2178,74	5379,68	2902,02	1787,65	2897,14	**3029,[illegible]**
P1U5	195,42	170,01	139,54	210,01	101,43	**163,28**	2049,95	1895,33	1888,49	2857,20	2605,37	**2259,[illegible]**
P1U9	104,22	137,09	227,42	298,87	192,62	**192,04**	3028,50	4168,03	4012,35	3588,92	2673,25	**3494,[illegible]**

KODE SAMPEL	KEDALAMAN KRIPTA					
	LP1	LP2	LP3	LP4	LP5	RERATA
P0U5	278,17	439,29	200,73	226,66	119,98	**252,966**
P0U7	432,69	361,95	458,53	502,33	279,25	**406,95**
P0U8	284,72	287,8	197,35	193,48	212,06	**235,082**
P1U1	223,74	167,96	281,31	310,16	150,68	**226,77**
P1U5	150,56	113,27	140,17	236,07	284,75	**184,964**
P1U9	246,34	182,61	156,08	220,51	187,8	**198,668**

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B. ILEUM

KODE SAMPEL	TINGGI VILI						LEBAR APIKAL					
	LP1	LP2	LP3	LP4	LP5	RERATA	LP1	LP2	LP3	LP4	LP5	RERATA
P0U5	1241,96	1014,61	1161,65	464,13	556,96	**887,86**	84,69	68,7	77,37	149,71	104,66	**97,03**
P0U7	893,43	854,25	897,11	843,55	887,01	**875,07**	48,25	53,09	63,03	58,1	76,06	**59,71**
P0U8	1200,2	1277,36	1422,03	1171,39	1176,59	**1249,51**	108,89	143,37	90,74	137,63	121,73	**120,47**
P1U1	917,84	1289,54	1385,58	1123,72	1023,06	**1147,95**	137,63	98,84	136,75	200,37	137,53	**142,2[illegible]**
P1U5	1311,16	1115,08	753,22	920,97	879,97	**996,08**	135,09	119,59	203,16	151,52	97,02	**141,2[illegible]**
P1U9	1467,1	1371,79	1418,18	1032,48	1246,7	**1307,25**	84,06	118,15	203,16	121,35	135,19	**132,3[illegible]**

KODE SAMPEL	LEBAR BASAL						LUAS VILI					
	LP1	LP2	LP3	LP4	LP5	RERATA	LP1	LP2	LP3	LP4	LP5	RERATA
P0U5	54,7	131,29	67,54	217,51	144,34	**123,08**	2044,12	2953,59	2175,71	1138,45	1325,08	**[illegible]**
P0U7	94,83	121,56	108,01	80,43	69,71	**94,91**	2649,37	2810,22	2434,42	2011,31	1699,97	**[illegible]**
P0U8	351,23	306,86	139,43	262,02	266,17	**265,14**	5071,50	4011,34	3607,10	3401,48	3749,28	**[illegible]**
P1U1	378,59	234,45	178,56	219,87	221,4	**246,57**	3442,62	4348,35	3194,79	2356,80	2670,01	**[illegible]**
P1U5	121,94	146,98	132,8	216,93	119,02	**147,53**	2494,69	2485,55	1245,58	2239,52	1959,48	**[illegible]**
P1U9	104,01	80,64	320,32	163,23	109,61	**155,56**	3282,39	2308,07	3654,21	2421,29	2257,51	**[illegible]**

KODE SAMPEL	KEDALAMAN KRIPTA					
	LP1	LP2	LP3	LP4	LP5	RERATA
P0U5	300,55	229,25	178,67	113,14	131,64	**190,65**
P0U7	202,34	181,53	211,56	245,85	183,41	**204,938**
P0U8	160,29	202,88	319,14	241,55	167,97	**218,366**
P1U1	283,06	236,97	148,16	299,74	258,73	**245,332**
P1U5	205,36	276,74	149,04	301,11	171,98	**220,846**
P1U9	326,87	206,44	247,32	125,34	256,57	**232,508**

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C. JEJUNUM

KODE SAMPEL	TINGGI VILI						LEBAR APIKAL					
	LP1	LP2	LP3	LP4	LP5	RERATA	LP1	LP2	LP3	LP4	LP5	RERATA
P0U5	935,47	1141,99	943,90	817,22	1392,40	**1046,20**	190,15	176,16	194,12	163,96	97,39	**164,36**
P0U7	988,86	898,12	1293,09	1012,05	766,03	**991,63**	75,54	100,48	69,34	74,66	95,79	**83,16**
P0U8	1106,58	836,44	1326,42	667,94	956,60	**978,80**	100,96	126,66	282,57	102,86	186,98	**160,01**
P1U1	1397,20	1370,85	1016,31	626,35	1290,75	**1140,29**	152,10	214,87	200,39	158,28	176,04	**180,34**
P1U5	1196,18	704,65	1080,71	1201,79	1360,53	**1108,77**	149,14	122,44	122,76	88,27	156,35	**127,79**
P1U9	1690,23	1739,65	1291,47	1007,93	922,47	**1330,35**	69,06	195,13	97,99	94,69	88,69	**109,11**

KODE SAMPEL	LEBAR BASAL						LUAS VILI					
	LP1	LP2	LP3	LP4	LP5	RERATA	LP1	LP2	LP3	LP4	LP5	RERATA
P0U5	382,05	168,03	372,79	138,58	179,37	**248,16**	2815,02	2231,28	2756,58	1507,94	3956,88	**2653,5[illegible]**
P0U7	216,47	225,25	279,14	112,17	154,74	**197,55**	3822,57	2911,47	6498,64	2532,56	2003,48	**3553,7[illegible]**
P0U8	206,86	135,9	209,63	165,52	174,38	**178,46**	3373,89	1733,90	2310,45	1742,77	1848,74	**2201,9[illegible]**
P1U1	368,68	134,59	132,86	235,71	345,87	**243,54**	4783,92	2229,52	1690,13	1559,11	3826,72	**2817,8[illegible]**
P1U5	219,86	256,09	125,71	101,15	142,63	**169,09**	2959,57	2178,46	2187,39	2578,94	2601,67	**2501,2[illegible]**
P1U9	227,98	236,6	221,51	182,09	194,16	**212,47**	7270,00	3849,02	4210,89	2946,19	2941,94	**4243,6[illegible]**

KODE SAMPEL	KEDALAMAN KRIPTA					
	LP1	LP2	LP3	LP4	LP5	RERATA
P0U5	143,23	283,52	178,12	164,03	155,46	**184,87**
P0U7	189,08	116,36	135,33	171,22	174,88	**157,37**
P0U8	175,69	167,61	11,41	189,67	202,74	**136,09**
P1U1	231,43	230,43	158,66	168,23	185,89	**194,93**
P1U5	182,25	146,17	191,41	348,05	252,15	**224,01**
P1U9	294,2	387,41	361,47	169,27	172,81	**277,03**

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CENTRAL RISET & DIAGNOSTIK
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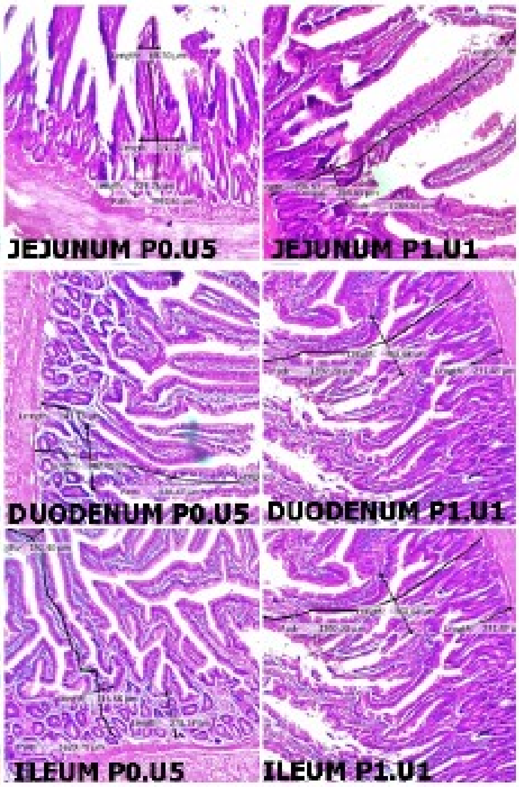
GAMBAR



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DELTA
HORSE
POWER

Delta Horse Power

Health Beneficial Effects of Peak Performance and Peak Performance Plus

Summary highlights

Delta Horse Power

Content

Introduction

Peak Performance and Peak Performance Plus are produced according to a proprietary recipe using a combination of different natural products that were traditionally used to enhance endurance, fertility and resilience. By blending the right ingredients and following a precise production process, we created a unique and powerful supplement.

The ingredients used in Peak Performance and Peak Performance Plus are sourced based on both traditional knowledge and contemporary scientific standards. These ingredients are selected according to the quality criteria established for medicinal and health-promoting products. The documentation of herbal health practices has been continuously updated and refined with advances in modern science and technology.

Literature proves the effects of the key ingredients on the gut microbiome and because of the bi-directional crosstalk between gut and brain, we know that all the links to different organs are facilitated by the microbiome.

In addition to their role in maintaining the intestinal environment, gut microbes are increasingly being recognized for their influence on other organs through their ability to communicate through several routes, for example by producing short chain fatty acids, neurotransmitters, histamine and compounds that modulate the immune system.

Study Design

In a unique study design, multiple complementary research angles were integrated to investigate the effects of Peak Performance and Peak Performance Plus on horses using methodologies to allow mapping of phenotypic, physiological as well as molecular effects.

1. The observed clinical effects were translated into molecular mechanisms that might be causal to these health benefits.
2. A metabolomics analysis was performed to enable large-scale mapping and quantification of the levels of all molecules involved in processes of metabolism influenced by Peak Performance and Peak Performance Plus.
3. A transcriptomics analysis was performed to enable large-scale mapping and quantification of all gene expression levels and profiles of genes influenced by Peak Performance and Peak Performance Plus.

A. CLINICAL OBSERVATIONS

Observed clinical effects by veterinarians, breeders, riders, and grooms:

CLINICAL OBSERVATIONS	
Weight	Trend towards increased weight
Coat condition	Increased shining coat in most horses, observed reduction in sensitivity to skin infection
Cardio respiratory function	trend towards reduced heart rate, normalized breathing when initially sensitive
Muscle volume	Observed increase

Blood cell count	Trend towards increased amounts of red blood cells, hematocrit and white blood cells
Relaxed behavior	Increased in almost all horses
Focus/concentration during exercise	Increased in almost all horses
Sweating after exercise	Reduced
Distraction during exercise	Reduced in most horses
Heart rate after exercise	Trend towards reduced heart rate after exercise.

B. PHENOTYPE MAPPING

The observed effects by equine specialists were translated into molecular mechanisms that might be causal to these health benefits. To link the phenotype observations to molecular processes, a data science approach was used. This analysis identified three pathways potentially involved with the phenotypic effects: 'DAG and IP3 signaling', 'Ketone body metabolism', and 'trans-Golgi Network Vesicle Budding'.

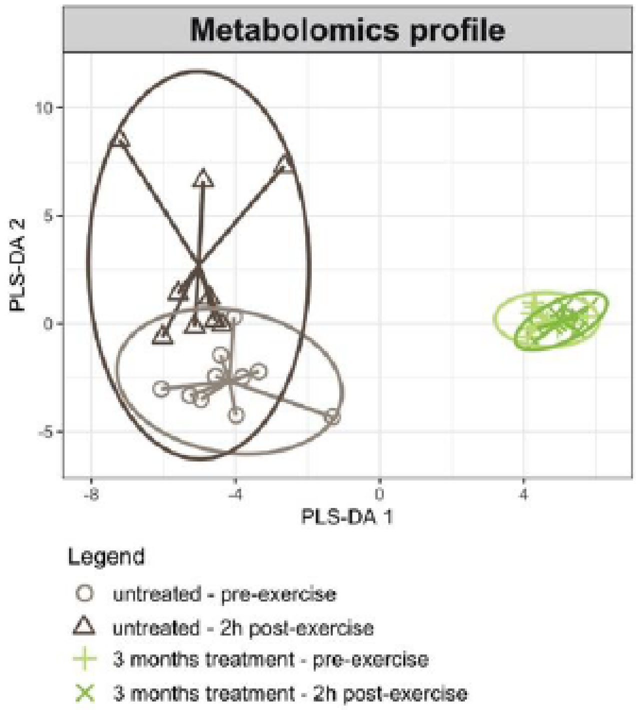
- DAG and IP3 signaling are important in signal transduction within cells, main function is mobilizing calcium from cells, this is an essential process involved in muscle contraction and neuronal signaling.
- Ketone body metabolism is important for maintenance of energy levels.
- Trans-Golgi Network Vesicle Budding is essential for transport of proteins.

C. METABOLOMICS

In untreated animals, there are quite some differences in metabolic profiles before and after exercise. In contrast, after three months of treatment, there are very little differences in the metabolic profile before and after exercise. This could indicate that animals are less metabolically affected by exercise after treatment, or potentially that their metabolism is more robust and can therefore better withstand the metabolic effects of exercise.

Highlight of the metabolomic findings are:

- Increase in amino acids and fatty acids and decrease in sugars is normal after exercise, these differences were less after three months treatment, this indicates that the horses were less affected by exercise.
- Decrease in cholesterol: High levels of cholesterol could indicate abnormal lipid metabolism.
- Tyrosinase: As a precursor to dopamine, the changed level of tyrosinase indicates a change in the cognitive physiology.



D. TRANSCRIPTOMICS

Transcriptomic profiles from blood of untreated horses were compared to animals after one and three months of treatment. From these profiles, the activity levels of 14281 unique genes were detected from blood samples.

When looking at overall changes in gene expression, we identified 5589 genes with a significant change in expression. The strongest enrichment was identified for processes related to brain functions. There was also a significantly changed gene expression activity related to heart contraction and lung development.

The expression of genes associated with reproduction and spermatogenesis was increased as well. Interestingly, we found an increase in expression of genes that code for collagens. This is of special importance as high levels of collagen aid in the prevention of injuries.

CONCLUSION

The comprehensive study on Peak Performance and Peak Performance Plus supplements reveals substantial benefits across multiple dimensions of equine health and performance. The phenotypic improvements observed, such as increased muscle mass, enhanced coat shine, and a more relaxed demeanor during exercise, underscore the positive impact of these supplements on the physical attributes of horses. Furthermore, these supplements contribute to a more resilient metabolism, as evidenced by a reduction in metabolic fluctuations post-exercise and an increased production of essential amino acids and fatty acids, indicating a more stable metabolic state.

On a molecular level, the study identified significant enhancements in gene expression related to critical biological functions. Notably, there was a marked increase in the expression of genes involved in brain activity, which could correlate with improved cognitive function and focus. Additionally, the upregulation of genes associated with heart contraction and lung development suggests that these supplements play a vital role in supporting cardiovascular and respiratory health, which are crucial for peak athletic performance.

The study also highlighted a significant boost in gene expression related to reproductive health, including spermatogenesis, indicating that Peak Performance and Peak Performance Plus may enhance fertility and reproductive outcomes in horses. The increased expression of collagen-related genes is particularly noteworthy, as collagen is essential for maintaining the structural integrity of tissues, which could lead to a reduced risk of injury and better overall durability in horses.

In summary, Peak Performance and Peak Performance Plus are not only effective in improving visible phenotypic traits but also in promoting deeper physiological and molecular enhancements. These supplements provide a scientifically backed, multifaceted approach to improving equine health, performance, and resilience, making them a valuable addition to the care regimen of any horse aiming for peak performance and optimal fertility.

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Beyond Just Another Supplement - Revolutionizing Equine Nutrition by doctor Oliver Riede.

From Nutrition to Innovation: What are Supplements and Their Evolution

In the domain of animal nutrition, a wide range of products is marketed as feed supplements, often not meeting the definition of traditional feed ingredients as set out by the European Union. The EU's regulatory framework, particularly through Regulation (EC) No. 767/2009, clarifies the classification of feed materials, including specific categories such as mineral feeds or feeds designed for particular nutritional purposes. These feed ingredients can further be integrated into more complex formulations, such as complete or complementary feeds. What is commercially referred to as a " feed supplement" can, within the legal context, mostly be more accurately categorized as a complementary or mineral feed. Additionally, the realm of feed additives falls under a separate regulatory umbrella, as determined by Regulation (EC) No. 1831/2003, requiring approval from the European Commission prior to market introduction. Despite these distinctions, the term "feed supplement" remains prevalent in industry jargon and will be used here to discuss Peak Performance®, which may appropriately be considered a complementary feed.

The Distinctive Features of Peak Performance®

The market for horse supplements is flooded with options promising nutritional support during periods of high stress such as training and competition. While many products offer a range of benefits for health issues ranging from muscle support to joint health, they often rely on broad, undifferentiated claims while lacking specific scientific evidence. In stark contrast, Peak Performance® stands as a beacon of innovation, founded on a solid basis of scientific research and the latest advancements in molecular biology. This strategic research effort has shed light on the unique mechanism by which Peak Performance® enhances the metabolic effectiveness of horses.

Peak Performance® distinguishes itself by not merely adding nutrients from the outside but by improving the body processes of the horse in such a way that it stimulates the production of building blocks essential for best performance This optimization enables the natural, balanced production of essential compounds, thereby avoiding the usual risks of over- or undernutrition associated with traditional products. It is crucial that Peak Performance® has been proven to stimulate the production of certain substances, including a specific type of collagen. This collagen, as identified in scientific studies, is currently not available in any other market supplement and underscores the unparalleled nature of Peak Performance®. Such innovation ensures that Peak Performance® is irreplaceable by other supplements, offering unique benefits that are currently not achievable through conventional means.

By utilizing the horse's intrinsic genetic potential, Peak Performance® facilitates the internal generation of vital compounds, thereby going beyond traditional supplementation methods. This approach not only improves key physiological functions such as lung capacity, cardiac efficiency, or collagen synthesis but also sets a new standard in the enhancement of horse health and performance.

In essence, Peak Performance® is at the forefront of innovation in equine nutrition. Its development, rooted in empirical evidence and confirmed by real-world efficacy, marks it as an indispensable tool in the pursuit of equine excellence. This strategic positioning significantly differentiates it from standard offerings in the supplement landscape, reinforcing its status as a groundbreaking advancement in the field.

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Doctor Oliver Riede.


BIOZAR
ORIGINAL
Rijk aan jodium
Voedingssupplement
Inhoud 250 ml
JODIUM ONDERSTEUNT HET ENERGIENIVEAU
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Rijk aan Jodium
www.biozar-original.nl
info@biozar-original.nl
JODIUM ONDERSTEUNT HET ENERGIENIVEAU

Biozar® Original is a unique cocktail where ancient knowledge comes together.

Our diet has changed enormously in the last 40 years. Fortunately, a mentality shift is underway. We pay more attention to whether our fresh vegetables, fruit, bread and meat are organic. We read labels better, do more sport and treat our bodies more consciously

Medicines as we know them today did not exist in the Middle Ages. Plants and herbs have been used in various forms as dietary supplements. This knowledge has been built up over time and passed on from generation to generation. This ancient knowledge has been preserved and written down in several pharmacopoeias1.

In Biozar® Original different plants, herbs and mosses from different parts of the world have been combined to support the immune system2. The herbs, plants and Irish moss are 100% natural. The secret of Biozar® Original lies not so much in the ingredients, but rather in the way they are composed and in the fermentation process that precedes them. Biozar® Original contains, among other things, fennel (Foeniculum vulgare), maca (Lipidium perivanum chacon), red algae/seaweed (Chondrus crispus) and pepper berries (Schisandra chinensis). Fennel has been considered a healthy vegetable/spice since ancient times. Trans-anethole and fenchone are particularly important. Another ingredient in fennel are the so-called flavonoids. Flavonoids are antioxidants. Fennel is also rich in potassium, calcium, iron and beta-carotene (provitamin A).

Maca is a root vegetable that grows high in the Andes. It is known for its high quality nutrients. Important bioactive components of maca (besides essential nutrients and fiber) are phytosterols, lignans, (aromatic) glucosinolates, isothiocyanates and unique macaenes, macamides and maca alkaloids.[4]

The red algae/seaweed comes from the Atlantic Ocean. The pepper berry is one of the oldest medicinal plants in Asia.

Biozar® Original is naturally rich in iodine. A teaspoon (3ml) contains 200% RI3 for an adult. Among other things, iodine is good for memory, concentration and keeps the mind clear. In addition, it activates the natural energy in the body, supports the thyroid gland and has a positive influence on the functioning of the nervous system

Biozar® Original is gluten-free and contains no flavor enhancers or colorings.

1 A drug book is an official manual that describes, among other things, the appearance and properties of a substance.
2 FSA ID 2055
3 The RI or reference intake is the legally established guideline for a balanced diet.
4 Ortho-Knowledge https://www.orthokennis.nl/nutrienten/maca

Biozar reviews

10

★★★★★

Fantastisch

Ik heb prostaat****** en mijn PSA was 22,9
En na 2 maanden Biozar was mijn PSA gezakt naar 16,1

Het AVL was zeer positief daarover

Groetjes Tobias Uilenbroek

Aanbevelen?

9/19/2025 Tobias Uilenbroek , Amsterdam

10

★★★★★

Biozar helpt voor chronische darmontsteking

Al een aantal jaren heb ik colitis ulcerosa (chronische darmontsteking). Ik loop bij de MDL arts en behandelingen gehad wat niet echt heeft geholpen. Toen ben ik Biozar gaan gebruiken en dat heeft wel resultaat voor mij. Ik heb nu minder last van mijn darmontstekingen. Ik ben tevreden over Biozar en kan het mensen aanbevelen met dezelfde klachten. Groet MarK

Aanbevelen? Ja

8/9/2023 Mark , IJsselstein

10

★★★★★

Biozar werkt goed tegen Acne

Al mijn hele leven heb ik last van Acne op mijn borst.

Sinds het gebruik van Biozar is Acne verleden tijd geworden.
Biozar is voor mij een geweldig werkend product.

Ik kan het dan ook aanbevelen

Aanbevelen? Ja

7/6/2023 Jan , Brunssum

10

★★★★★

Kan en wil niet meer zonder

Sinds ik het gebruik heb ik veel minder last van mijn artrose en kan daardoor weer veel meer doen.

Aanbevelen? Ja

6/29/2023 john , Mijnsheerenland

10

★★★★★

Goed prodcut, top service!

First of all, wat een geweldige service bij Biozar Original, hier mogen andere bedrijven een voorbeeld aan nemen! Nadat ik via een andere verkoper op Bol.com Biozar Original had aangeschaft rook het product helemaal niet goed en zag ik dat de houdbaarheidsdatum nabij was. Viavia is mijn verhaal terecht gekomen bij team Biozar (shout-out to Hendrik D. die mij heeft geïntroduceerd aan het product en uitvoerig heeft verteld over de geweldige werking ervan).

Team Biozar heeft contact met me opgenomen en me onmiddellijk en kosteloos nieuw/vers product opgestuurd! Mijn complimenten voor deze geweldige, klantvriendelijke service, zo behoud je klanten!

Tot nu toe ben ik tevreden met het gebruik van Biozar, ik gebruik het minder dan een week dus ik kan nog niet veel zeggen, behalve dat mijn huid opklaart. Ik heb Biozar aangeschaft i.v.m. overmatige haaruitval, waar ik al jaren last van heb. Ik verzorg mijn haar goed, maar het blijft uitvallen- en dat kan niet als je een onderneming hebt die handgemaakte haarverzorgingsproducten verkoopt. Ik zal in de toekomst terugkomen voor een vervolg review, maar tot zover een 10! Keep on doing great guys!

Aanbevelen? Ja

6/12/2023 Farida Eintou , Utrecht

10

★★★★★

Van zombie naar levend

Via Volg je hart blog van Martijn Vis op de Biozar geattendeerd.

Al maanden kwam ik met alleen wilskracht uit bed en naar het werk (voelde als een zombie).
Nu: neem in de ochtend Biozar, doe ademhalingsoefeningen, kleine meta meditatie daarna paar zonne groetjes, koud douchen....

En ik knal van het leven en heb er weer zin in!

Ik drink het met Roosvice ijzer en heb geprobeerd om alleen Roosvice te drinken maar dit werk niet.

Beveel het iedereen aan, mijn man heeft er ook erg veel baat bij.
Onze zoon niet.

Dus misschien niet voor iedereen maar probeer het!

Dank jullie voor het maken van deze wonderlijke drank!!

Aanbevelen? Ja

6/7/2023 Miranda , Rotterdam

10

★★★★★

Topprodukt

Boost voor het immuunsysteem

Aanbevelen? Ja

4/16/2023 Jo , Leeuwarden

10

★★★★★

so far full success

We used Biozar to treat our dog's skin illness. The vet had identified this as the consequence of the immune system's overreaction. Cortisone was applied without any success. Applying Biozar, a positive reaction occurred after 6 days. We still continue with the treatment. Hopefully poor doggie will recover completely.
Your administration service, too, is *****.
Thank you very much.

Aanbevelen? Ja

4/7/2023 Brigitte , Portugal

10

★★★★★

Biozar werkt

Al jaren heb ik heupklachten.
Van alles geprobeerd maar niks hielp. Tot dat ik via via te horen kreeg over Biozar.

Sinds het gebruikt van Biozar zijn mijn heupklachten nagenoeg over.
Ik ben dan ook zeer te vreden over Biozar en raad het iedereen aan.

Hartelijke groet Helga

Aanbevelen? Ja

4/6/2023 Helga , Brunssum

10

★★★★★

Mijn kat fit en gezond

Beste,

Ik heb jullie bijna 2 jaar geleden gesproken voor mijn werk in de media destijds. Ik heb research van jullie mogen lezen mbt de positieve resultaten op onbewerkbare landbouwgrond, ernstig met olie vervuilde stilstaande wateren die binnen 24u helder waren, en uiteraard over de positieve effecten voor gezondheid mens en dier.

Mijn kat werd rond die tijd gediagnostiseerd met lymphklier****** en kreeg geen beste vooruitzichten van verschillende artsen. Ik heb bijna per direct na de interessante gesprekken met jullie Biozar besteld en geef het sindsdien in zijn drinkwater, het gaat nogsteeds super met hem.. Ik heb echt het idee dat het door Biozar komt dat hij nog steeds gezond is, ondanks de uitslag van de verwijderde tumor agressieve cellen bleken te zijn.

Het betreurt mij dat dit geweldige product zo tegengewerkt is door de chemische industrie die in o.a. landbouw en farmaceutische wereld door de overheid gesteund worden, in plaats van de producten die échte positieve veranderingen in deze wereld kunnen maken.

Hoop dat jullie verder zullen gaan met dit product promoten en zoveel mogelijk mensen hier kennis mee laten maken, ik vertel erover aan ieder die meer wil weten.

Met vriendelijke groet

Aanbevelen? Ja

12/15/2022 Demi van Elsland , 'S-GRAVENHAGE

10

Ik voel me fitter

Reeds enkele maanden gebruik ik Biozar. Ik voel met fitter en ben in staat meer te presteren dan voorheen. Ik ben zeer te spreken over Biozar en ben blij dat dit natuurlijke product bestaat.

Aanbevelen? Ja

8/23/2022 Dennis , IJsselstein

10

Raad het echt aan, ben 76 en neem elke dat 2 theelepels biozar

Ik voel mij fit en mijn hoge bloeddruk is heel erg gezakt. Mijn haar groeit sneller en mijn nagels ook.

Dank je wel

Aanbevelen? Ja

2/12/2022 Helen van Dijk de Klerk , Amsterdam

9

Mijn ervaringen zijn

Met Biozar was er een als een versterking van mijn immuunsysteem. Maar later bleek dat het mijn pacemaker overbodig had gemaakt . Ik droeg +/- 10jaar een pacemaker toen ik Biozar ging gebruiken , bij een controle een half jaar later werd mij verteld dat de pacemaker mij hart niet meer had ondersteund . 8 jaar later is de pacemaker verwijderd hij was al jaren overbodig .

Helaas had ik ook nog een hypofyse adenoom van +/- 4 cm die is nu 5 jaar geleden verwijderd voor alle zekerheid , ondanks dat de tumor nagenoeg niet was gegroeid . Nu 4 maanden geleden een scan gehad en de restanten van de tumor waren niet gegroeid . Ik heb het volste vertrouwen in Biozar . En bij deze heel hartelijk dank Theo .

Aanbevelen? Ja

2/8/2022 Jan , Berlikum

10

★★★★★

Zo blij dat Biozar op mijn pad is gekomen

Oktober 2020 werd mijn zus gediagnostiseerd met uitgezaaide nier******. Een second opinion bij het Anthonie van Leeuwenhoekziekenhuis leverde dezelfde diagnose op. De oncoloog vertelde mijn zus, ik kan je niet beter maken, we kunnen enkel proberen je tijd van leven enigszins te rekken, middels chemokuren. Via een vriend van mij hoorde ik over Biozar, een middel dat ondermeer op basis van planten-extracten van over de hele wereld je eigen immuun-systeem zodanig een boost geeft, dat je eigen lichaam de ******cellen opruimt. Mijn zus is Biozar gaan gebruiken. Ze heeft tegelijkertijd ook een tijdje chemokuren gehad, maar de chemokuren heeft ze halverwege moeten staken, omdat ze er zo ontzettend ziek van werd dat ze zelfs meerdere keren ernstig verzwakt in het ziekenhuis belandde. Na een tijd kreeg mijn zus een CT scan en tot verbijs****** van de oncoloog was de tumor in haar nierbekken enorm veel kleiner geworden en de door de ****** vergrootte lymfeklieren in haar buik waren ook enorm geslonken. Bij de laatste scan was er geen tumorweefsel meer te zien op de CT scan. Ik had ook over Biozar gesproken met een goede vriend, die huisarts is in Delft. Hij heeft Biozar genoemd tegen een patiente van hem met uitgezaaide slokdarm******. De vrouw heeft Biozar via internet besteld en is het gaan gebruiken. Nu is het een jaar later en leeft ze nog steeds en op de laatste scan was er geen ****** meer te ontdekken, aldus mijn vriend de huisarts. Ik ken ook mensen die het niet willen proberen, omdat de reguliere geneeskunde het niet voorschrijft. De reguliere geneeskunde leunt geheel op de farmaceutische industrie, maar dat betekent niet dat Biozar geen middel is dat stoelt op wetenschappelijke inzichten, durf ik als wetenschapper (chemicus) te stellen. Biozar heeft ook als voordeel dat het geen bijwerkingen heeft zoals bijvoorbeeld chemo-kuren. Mijn zus zei: ik raad Biozar iedereen aan !

Aanbevelen? Ja

2/6/2022 Hendrik , Aalsmeer

10

★★★★★

Biozar geeft mij energie

Ik heb al tien jaar chronische lymfatische leukemie en ben naar een jaar biozar gaan gebruiken waarna mijn bloedwaarden dusdanig verbeterd waren zodat ik geen chemotherapie nodig had. Nu negen jaar later zijn mijn bloedwaarden nog steeds stabiel en voel ik mij prima. Dus ik raad iedereen met ****** aan om biozar te gaan gebruiken en hoopt dat deze mensen net als ik zonder andere medicatie zich ook gelukkig en fit voelen door het gebruik van dit wondermiddel!

Aanbevelen? Ja

2/4/2022 Wil , Rhenen

10

Super booster!

Mijn vader heeft ****** en gebruikt Biozar nu een paar weken, maar ik zie hem vooruitgaan! Meer energie, meer eetlust! Wat fijn om te zien.

Aanbevelen? Ja

1/29/2022 Sandrina , Limmen

10

Ben zo blij

Heb vijf jaar eczeem, na twee weken is het echt al bijna weg. Meer energie en haar groeit sneller.

Aanbevelen? Ja

10/11/2021 Ellen , Amsterdam

10

prima spul van Biozar.

Het is wonderlijk ik gebruik het nog niet zolang maar de waardes in het

ziekenhuis zijn sterk verbetert ik heb prostaat ****** met een uitzaaing EN DE NIEREN WAREN OOK NIET IN ORDE VOLGENDE WEEK WEER VOOR CONTROLE NAAR HET ZIEKENHUIS WE ZULLEN ZIEN MAAR IK HOOP GENEZING/?

Aanbevelen? Ja

10/7/2020 DAAN , ALPHEN AAN DEN RIJN

Media

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Thank you

Wiseuse International Message of Gratitude and Invitation

Wiseuse International would like to thank all its partners, volunteers, universities, and students for their professionalism, extraordinary work, and dedication.

Keep up the great work!
Wiseuse warmly invites everyone who feels inspired to join our research and projects—because innoation never stops.

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