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CYANOBACTERIA IN FRESHWATER: INFLUENCE IN LIVER MORBIDITY IN EXPOSED ALENTEJO'S POPULATION BETWEEN 2000 AND 2010

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Keywords: *cyanobacteria; toxins; Liver morbidity; Liver cancer.*

Abstract

Taking nutrients from the water column, phytoplankton can present high levels of growth and blooms arise if growth rates reach densities over 2.000 cells/ml. Cyanobacteria (prokaryotic, photosynthetic microorganism) is one group of this community and can produce toxins responsible for human injury after acute or systematic exposition.

The main goal of this work is to correlate cyanobacteria blooms in Alentejo reservoirs and liver morbidity (cancer disease) in human exposed population, through direct or indirect water consumption.

In Portugal seven reservoirs used to produce drinkable water located in Alentejo region were selected and studied between 2000 and 2008. Reservoirs were characterized in physical and chemical aspects, as well as phytoplantonic communities, through identification and counting of main present groups along the study period**.** In expressive blooms circumstances, liver toxin producers were founded, namely *Microcystis aeruginosa*, *Aphanizomenon spp*and *Oscillatoria*, and liver condition indicators showed highest levels in exposed populations. Liver cancer incidence was also more expressive in the exposed population, compared to non-exposed population. It was concluded that cyanotoxins systematic exposition promotes liver morbidity in the exposed population, attested by high levels of liver enzymes, cancer disease incidence and relative and attributable risks values, compared to non-exposed population's values.

Introduction

Water ecosystems productivity is supported by phytoplankton community that use light, Carbon dioxide and organic nutrients to produce biomass (Paerl et al., 2001).These photosynthetic organisms present different needs and answers to physical and chemical aspects according species, and several groups like Cyanobacteria, Chlorophyceae, Chrysophyceae, Bacillariophyceae, Cryptophyceae, Dinophyceae, Euglenophyceae and Conjugatophyceae (Lee, 2008) can be referred. Cyanobacteria (or blue algae) are prokaryote, photosynthetic organisms, and the only group able to fix atmospheric nitrogen or environment Phosphorus and use it in environment scarcity circumstances.(Paerl et al., 2001).

1. Phytoplankton Blooms

Taking nutrients from the water column, phytoplankton can present high growth levels and phytoplankton blooms (algae/cyanobacteria) arise if growth rates reach densities over 2.000cells/ml. These blooms are monitored with biomass measures – cells counting/ml, cell bio- volume (mm^3/L) – associate to found species.

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2. Toxic Blooms

When blooms occur, concerns are related to phytoplankton groups like Cyanobacteria, Bacillariophyceae or Dinophyceae, because they can promote toxicity in aquatics and terrestrial animals, trough toxins production. These toxins can remain inside cells and being released during active cell growth or cell lysis(Camargo & Alonso, 2006). Among toxin producers, cyanobacteria is the most notorious group in fresh waters including Nitrogen fixing genders (*Anabaena, Aphazinomenon, Cylindrospermopsis, Gloetrichia and Nodularia*), and non-fixing Nitrogen genders (*Microcystis, Oscillatoria and Lyngbya*) (Paerl et al., 2001). Human intoxication is associated with contaminated and improperly treated water ingestion or recreation activities (Dias, 2009).

3. Cyanotoxins

Cyanotoxins are secondary metabolites formed from pigments (Santos & Bracarense, 2008), and they assemble several groups of toxins (Svircev et al., 2009). Each cyanotoxin can be produced from several cyanobacteria species and one specie can produce more than one kind of toxin. According toxicological profile, they can be grouped as hepatotoxic, neurotoxic and cytotoxic (Funari & Testai, 2008) among others .

3.1 Hepatotoxic Cyanotoxins

3.1.1 Microcystins

They are the most dangerous group of toxins and *Microcystis, Planktothrix and Anabaena*are dominant producer genders (Falconer & Humpage, 2005). This toxins can resist to the digestion process in Eukaryotes and concentrate in liver by active transportation, where Phosphatases 1 and 2A can be inhibited. This enzyme control several cellular processes and their inhibition can cause changes in cell division and promote tumors formation (Barbosa, 2009), (Falconer & Humpage, 2005).

Liver injury through destroyed cells can be detected with raised blood levels of liver enzymes such as Aspartate Transaminase (AST), Alanine Transaminase (ALT), Gamma Glutamyl Transpeptidase (GGT) or Alkaline Phosphatase (ALP) (Pádua, 2009). According Moss and Henderson (1987), GGT is the most sensitive indicator of liver disease but, raised levels can also be associated to alcohol intake or alcohol cirrhosis.

In addition to liver injury, several epidemiological studies suggest responsibility of cyanotoxins in high primary liver cancer incidences, recorded in studied populations (Barros, Souza, Tavares, & Amaral, 2009; Damjana et al., 2011; Hernández, López-Rodas & Costas, 2009; Palus et al., 2007; Zegura et al., 2011).

3.1.2 Nodularin

This liver toxin is produced by cyanobacteria *Nodularia spumigen* and acts the same way as *microcistin group.*

3.1.3 Cylindrospermopsin

Is produced by *Cylinderospermopsis raciborskii, Aphanisomenon ovalisporume and Umezakia natans*(Haider et al., 2003) and is very toxic to hepatic cells rodents by protein synthesis inhibition (Falconer & Humpage, 2005).

4 Cyanobacteria in Portugal

Cyanobacteria in natural lakes and reservoirs have been reported in Portugal since the 30s but, toxic cyanobacteria distribution studies started only after 1989 (Vasconcelos, 1999). Between 1989 and 1992, 30 lakes, rivers and reservoirs have been studied and 18 bloom samples revealed toxin presence. Dominant cyanobacteria species were *Microcystis. aeruginosa, Microcystis wesenbergii, Anabaena flos-aquae, Anabaena scheremetievi Aphanizomenon flosaquae and dominant toxin Microcistin LR*. According Araújo (1995) several results revealed cyanobacteria blooms in reservoirs used to produce drinkable water in Alentejo, and that was a potential risk to public health because there was not water treatment to eliminate cyanotoxins.

The main goal of this work is to correlate cyanobacteria blooms in Alentejo reservoirs and liver morbidity (cancer disease) in human exposed population, through direct or indirect water consumption.

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As specific goals can be cited:

I) Physical, chemical and phytoplankton water features in studying Alentejo reservoirs, potential toxicity produced by cyanobacteria, and identification of critical contamination periods;
II) Describe the demographic evolution of residents in reservoirs regions
III) To compare rates of water consumption between exposed and non-exposed inhabitant;
IV) To compare liver morbidity indicators between exposed and non-exposed inhabitants;
V) Describe chronically infectious liver morbidity indicators (B and C Hepatitis) in studying populations;
VI) To compare incidence liver cancer between exposed and non-exposed population;
VII) Evaluate relative risk in population associated with cyanobacteria toxins continued exposure through water consumption;
VIII) To evaluate attributable risk in population caused by cyanobacteria toxins continued exposition through water consumption.

Materials and Methods

1. Characterization of study population

Exposed and non-exposed population resident in Alentejo (Portugal South-central region), in particular population living in Beja and Évora districts, where studied reservoirs are located.

2. Characterization of study region (reservoirs)

Seven reservoirs located in Alentejo (South Portugal) and referenced to produce drinkable water were selected:

i) from Sado and Mira watershed: Alvito (14,8Km^2) Roxo (13,7 Km^2) and Monte da Rocha (7,640 Km^2);
ii) from Guadiana watershed: Boavista (3,5 Km^2), Enxoe (2 Km^2), Monte Novo (2,7 Km^2) and Vigia (2,6 Km^2);

According Koppen classification, clime is Mediterranean, characterized by hot summers and rainy winters(Csa) (Morais et al. 2003)

3. Physical and chemical characterization of reservoirs

Physical and chemical data between 2000 and 2008 were collected from the National System for Water Management Information discontinued database: Alvito (2000 till 2005), Roxo (2000 till 2008), Monte da Rocha (2003 till 2005), Boavista (2006/2007) Enxoé (2003/2004), Monte Novo and Vigia (2000 till 2007).

4. Phytoplankton characterization

Phytoplankton evaluation was made with data provided by water laboratory from Évora University. The data referred to identification and quantification of organisms belonging to each taxon, were obtained by Utermohl Method (1958), and bio volume calculation. Using molecular absorption spectrophotometry and Lorenzen equation (American Public Health Association, 2000), Chlorophyll *a* concentration was also estimated.

5. Population data

All population data were selected from Portugal Census 2011, and provided by the Statistic National Institute

6. Health population data

Health data (liver function indicators, hepatic viral infection, alcohol liver disease and liver cancer incidence) were provided by Public Institutions (Statistic National Institute, South Region Oncologic Record and Hospitals)

Data processing

Each set of data was initially processed with descriptive statistics to identify central tendency measures, dispersion measures and graphic representations. To compare health data, water consumption rates and incidence liver cancer rates in exposed population and non-exposed population, Mann-Whitney test was used. To calculate relative and attributable risk, epidemiological formula was applied:

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Relative Risk (RR) = Exposed population incidence rate / Non-exposed population incidence rate

Attributable Risk (AR) = (Exposed population incidence rate) - (Non-exposed population incidence rate)

Total Population Attributable Risk (TPAR) = (Total population incidence rate) - (Non-exposed population incidence rate)

Statistical Package for the Social Sciences (SPSS) and Excel (2010) were used to do analysis and graphical representations.

Results and Discussion

1. Physical parameters

Temperature, pH and Conductivity

Reservoirs presented temperature average values between 18,1° C and 20,1° (Sado watershed) and 18,8° C and 19,3° C (Guadiana Watershed)**.**Guadiana watershed reservoirs (Boavista and Monte Novo) presented highest pH values, but all other reservoirs presented pH average values over 7. Highest conductivity was recorded in Roxo reservoir, probably related tosalt contamination from industries and agriculture effluents **(Table 1)**. According Carvalho, Schlittler, e Tornisielo (2000), temperature rise and suspended solid can also promote conductivity rise.

Table 1: Average and Standard Deviation Physical values

Watershed		Sado			Guadiana			
Reservoirs		Alvito	Mt^{e} Rocha	Roxo	Boavista	Enxoé	M^{te} Novo	Vigia
Evaluation period (2000/2008)		2000/05	2000/05	2000/08	2006/07	2003/04	2000/07	2000/07
Parameters	RMV* AMV**							
Temperature (°C)	22* 25**	18.6 SD 4.7 N = 72	20.11 SD4.8 N=66	18.1 SD 4.2 N= 120	19.3 SD 4.8 N=23	19 SD 4.8 N=25	18.8 SD 5.1 N=92	18.7 SD 5.4 N=98
pH (25° C)	5.5 – 9.0*	8.1 SD 0.2 N=72	7.8 SD 0.9 N=19	8.1 SD 0.2 N= 106	8.2 SD 0.2 N=24	8.1 SD 0.2 N=25	8.2 SD 0.4 N=94	7.8 SD 0.5 N=96
Conductivity (µS/cm, 20° C)	1000 **	302.7 SD 36.3 N=71	266.5 SD 46.9 N=69	1064. SD 182.3 N=107	239.3 SD 34.7 N=24	467.0 SD 55.6 N=25	350.8 SD 56.0 N=94	275.4 SD 63.2 N=95

* Maximum Recommended Value
** Maximum Allowed Value

2. Chemical parameters

Nitrogen components, Solved Oxygen, Biochemical Oxygen Demand and Manganese

Some of studied chemical parameters (Solved Oxygen, Ammoniacal Nitrogen, and Manganese) presented average values above recommended limits. Other like phosphates and nitrates keep lower average values(**Table 2**),Monte da Rocha reservoir presented highest nitrogen component value (Ammoniacal Nitrogen) and Alvito reservoir the lowest value. All reservoirs presented Solved Oxygen values above recommended limits (in particular Alvito reservoir – 99%) and this condition, may be connected to high primary productivity. Biochemical Oxygen Demand average values

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approached recommended values indicating organic matter rise. Usually associated to industrial discharge, Manganese presented average values equal or greater than recommended values.

Table 2: Average and standard deviation values for studied chemical parameters

Watershed		Sado			Guadiana			
Reservoir		Alvito	Mt^{e} Rocha	Roxo	Boavista	Enxoé	M^{te} Novo	Vigia
Evaluated period (2000/2008)		2000/05	2000/05	2000/08	2006/07	2003/04	2000/07	2000/07
Studied parameters	MRV* MAV**							
Solved Oxygen (%)	50*	99 SD 22.9 N=70	78.9 SD 15.9 N=61	85.1 SD 20.0 N=115	86.1 SD 14.6 N=20	79.8 SD 22 N=25	92.2 SD 19.3 N=86	71.7 SD 28.4 N=91
Ammoniacal Nitrogen (mg/L NH_4)	1.00 * 1.50**	0.05 SD 0.0 N=71	1.04 SD.04 N=31	0.16 SD 0.14 N=105	0.09 SD 0.04 N=24	0.35 SD 0.5 N=25	0.12 SD 0.07 N=94	0.16 SD 0.10 N=95
Nitrates (mg/LNO_3)	50*	0.77 SD 0.4 N=72	0.86 SD 1.9 N=70	1.4 SD 0.9 N=105	1.8 SD 1.3 N=24	0.68 SD 0.36 N=25	1.1 SD 0.8 N=95	2.1 SD 1.3 N=96
Phosphates (mg/LP_20_5)	0.7*	0.03 SD 0.02 N=71	0.05 SD 0.04 N=69	0.06 SD 0.03 N=107	0.1 SD 0.04 N=24	0.14 SD 0.04 N=25	0.07 SD 0.03 N=95	0.06 SD 0.03 N=85
Phosphates (µg P/L)	700	30	50	60	100	140	70	60
Manganese (mg/L Mn)	0.10*	0.07 SD 0.07 N=37	0.09 SD 0. 13 N=34	0.04 SD 0.04 N=50	0.1 SD 0.07 N= 12	0.10 SD 0.03 N=12	0.06 SD 0.04 N=48	0.18 SD 0.24 N=49
Biochemical oxygen Demand- CBO_5 (mg/L O_2, 20° C)	5*	3,0 SD 1.0 N=67	4.4 SD 2.7 N=64	3.8 SD 1.1 N=97	3.0 SD 0.0 N=20	4.6 SD 1.2 N=25	3.6 SD 0.9 N=85	3.9 SD 1.6 N=86
Total Nitrogen (mg/L) (Kjeldhal+Nitrates)		1.3 SD 0.5 N=71	ND*	2.3 SD 0.9 N=105	2.4 SD 1.4 N=24	2.7 SD 0.6 N=25	2.2 SD 0.8 N=94	3.2 SD 1.5 N=95
Ratio TN/TP		43	---	38	24	19	31	53

* Maximum Recommended Value
** Maximum Allowed Value

Registration temperatures above 15° C may have promoted cyanobacteria grow and moderate alkalinity can be explained by photosynthesis activity related to blooms, as referred by Havens (2008).

Nitrogen and Phosphorus levels weren't high, but this condition did not avoided cyanobacteria grow because same gender can fix atmospheric Nitrogen (*Anabaena, Aphanizomenon ou Cylindrospermopsis)* or store Phosphorus (Havens et al. 2003), (Chorus and Bartram 1999).

3. Reservoirs Trophic Condition (2000 -2008)

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Six reservoirs were classified as eutrophic systems, four of them because average Chlorophyll-*a* value was higher than 10 µg/L (**Figure 1**), and two of them because Total Phosphorus average was over 35µg P/L (**Figure 2**)

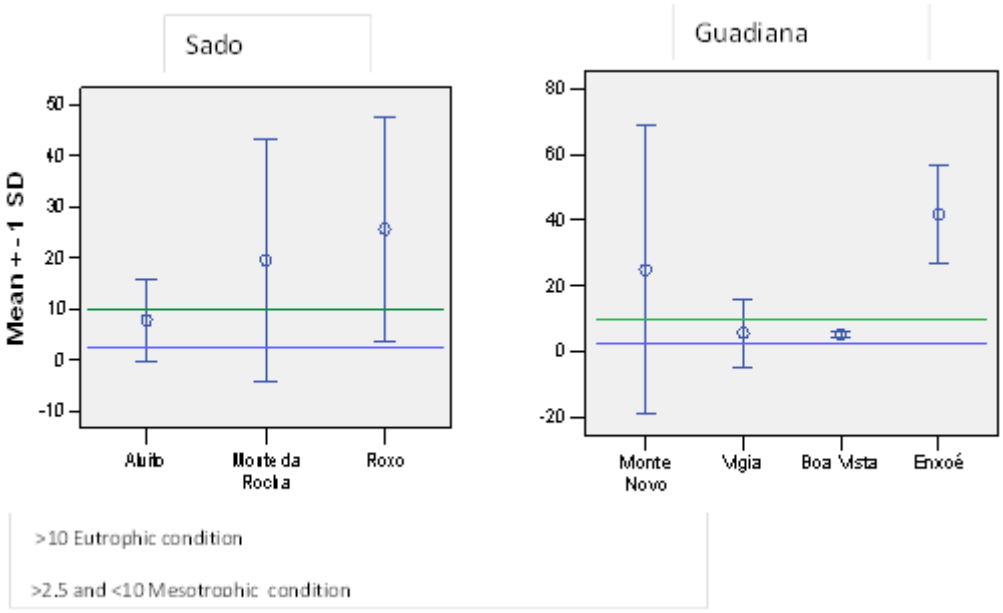

Figure 1: Chlorophyll-a average values (µg/L)

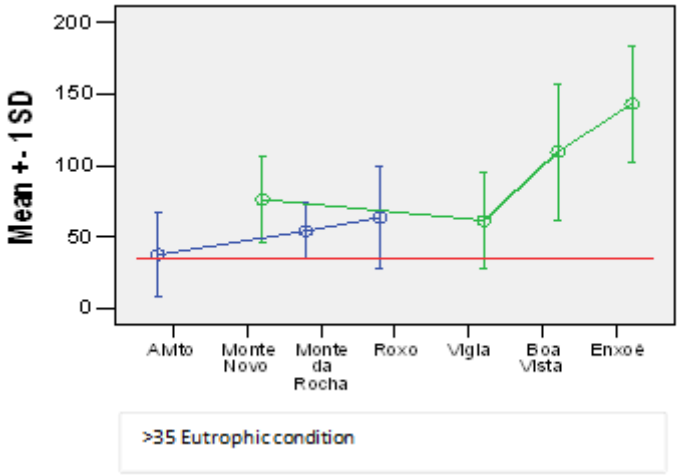

Figure 2: Total Phosphorus average values

Total Phosphorus geometric average above 35 µg/L recorded in studied reservoirs can be explained by point and diffuse contamination sources that contributed to water eutrophication condition as referred by Coelho and Leitão (2001).

All studied reservoirs presented cyanobacteria blooms during evaluated period (one of them presented more than 1.200.000 cells/ml), and registered liver toxin producers (**Table3**) in most intense blooms.

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Table 3 Liver toxin producers in most expressive cyanobacteria blooms

Reservoir	Simple	Cells/ml - Cyanobacteria	Toxic properties
Alvito	Winter 2004	327.455 *Snowella sp.* 3.639 - *Microcystis sp.* 2.812 - *Aphanizomenon sp.*	Annoying toxin Liver toxin Liver and NS* toxin
Boavista	Autumn 2007	1.270.173 - *Microcystis aeruginosa*	Liver toxin
Enxoé	Autumn 2003	817.530 - *Aphanizomenon sp.* 12.644 - *Oscillatoria sp.*	Liver and NS* toxin Liver and NS* toxin
Monte da Rocha	Autumn 2004	253.226 *Aphanocapsa sp.* 13.824 - *Microcystis* sp.	Annoying toxin Liver toxin
Monte Novo	Spring 2006	114.595 *Microcystis sp.*	Liver toxin
Roxo	Summer 2005	320.642 Oscillatoria *sp.* *32.504 – Planktothrix sp.*	Liver and NS* toxin Liver and NS* toxin
Vigia	Spring 2007	183.593- *Woronichinia sp.* 6.213 - *Anabaena sp.* 7.950 - *Aphanizomenon sp.* 17.095 - *Microcystis sp.*	Annoying toxin Liver and NS* toxin Liver and NS* toxin Liver toxin

*Nervous System
Adopted from: Chorus & Bartram (1999), Global Water Research Coalition (2009a), Bellém, Nunes & Morais,(2013).

3. Demographic evolution and water consumption pattern (2001 – 2010)

During this period, both districts (Beja and Évora) presented same trend, keeping the percentage of individuals between 5 and 14 years old, and decrease young population (15 to 24 years old), in both genders. The percentage of people over 45 years old raised, especially above 75 and male gender. With decrease of a young population and raised middle age and old people, we can conclude about the general aging of the studied population.

Between 2001 and 2009 water consumption pattern was kept in this population, with the highest consumption levels in the exposed population, compared with non-exposed population (**Figure3**).

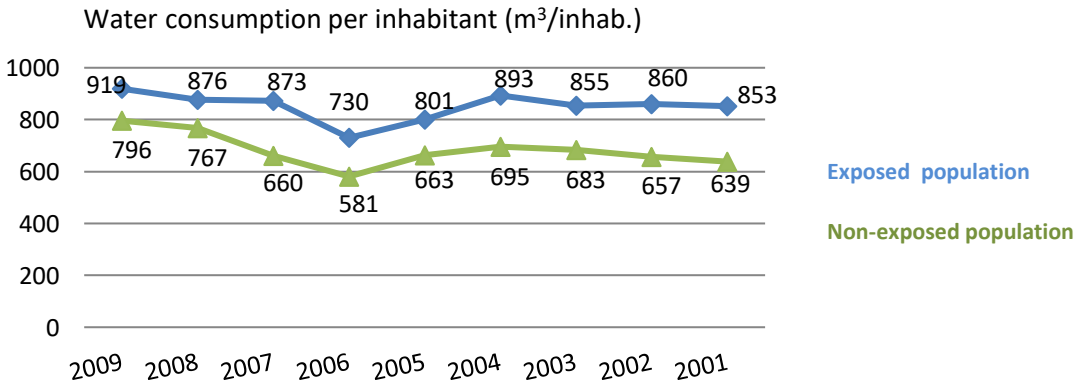

Figure 3: Water consumption evolution (2001 – 2009)

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4. Liver morbidity Indicators (2004 – 2010)

To evaluate liver morbidity in exposed and non-exposed population, 3enzymes (AST, ALT and GGT) serum levels were considered. AST and GGT enzymes were assessed between 2004 and 2010 but for ALT enzyme only 2009 and 2010 data were available. With the exception of the year 2004, AST and GGT average values were highest in exposed population when compared to non-exposed population (**Figure4**) but, both level enzymes decrease between 2009 and 2010.

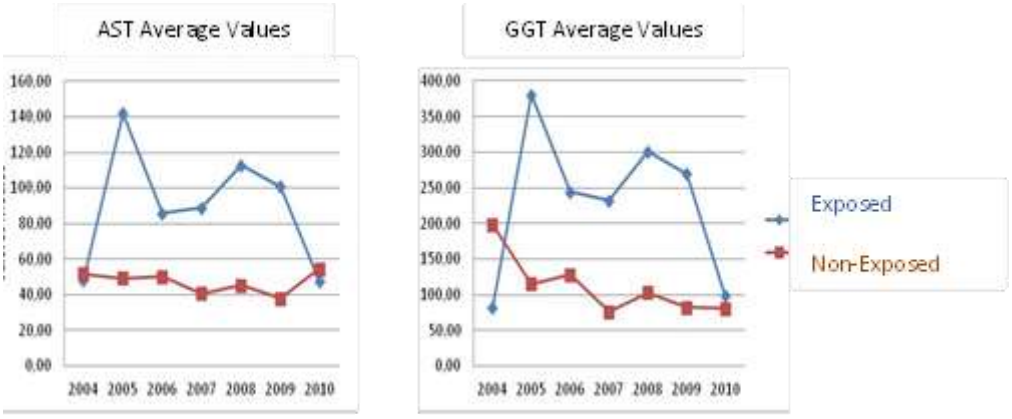

Figure 4: AST and GGT average values by exposition type

The ALT enzyme also presented highest levels in the exposed population (**Table 4**) but, levels decreased in 2010 compared with 2009.

Table 4: ALT average values in 2009 and 2010

	Exposed		**Non-exposed**	
	GPT-ALT 30-65*		GPT-ALT 30-65*	
Years	Evaluations N	Average values	Evaluations N	Average values
2009	1234	104,1	6278	36,9
2010	21150	54,8	5728	50,8

*Reference values

To compare enzyme serum levels in exposed and non-exposed population, non-parametric Mann-Whitney test was used and some significant statistic differences were found (P<0, 05 and P<0, 01) (**Table 5**).

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Table 5: Liver enzymes statistic differences

	2004/05	2006	2007	2008	2009	2010
	AST		GGT		ALT	
	Mann-Whitney U	p	Mann-Whitney U	p	Mann-Whitney U	p
2004	27754,000	0,013**	6903,500	0,000*	--	--
2005	15019548,500	0,000*	4793759,500	,778	--	--
2006	4179032,000	0,000*	1276484,500	0,000*	--	--
2007	27081293,500	,683	9944927,500	0,000*	--	--
2008	13054082,000	,863	5112974,500	,060	--	--
2009	63079078,000	,349	22659231,000	,059	62447421,500	0,012**
2010	59132774,500	0,000*	20687020,500	0,000*	57433063,000	0,014**

* Significant statistic differences (1% level of significance)
** Significant statistic differences (5% level of significance)

Comparison between exposed and non-exposed population showed highest liver enzyme concentration on the first group (exposed), meeting results presented by Chen, Li & Xu (2009) and Araújo (1995).

Those enzymes different values indicate liver commitment connected to cianobacterial toxins continuous exposition through drinkable water, as suggested by Araújo (1995), Moreno et al., (2003) and Svircev et al., (2009).

Isolate GGT enzyme levels can also increase by alcohol or medicines ingestion(Pádua, 2009). In order to decrease this influence in results analysis, percentage of raising GGT levels in individuals aged between 20 and 50 years old (more prone to alcohol intake) were verified. This percentage decreased between 2004 and 2010 (**Table 6**)

Table 6 - Percentage of individuals aged between 20 and 50, with isolated GGT raised value

	Value%	31%	23%	20%	20%	13%	12%
	N	420	446	437	414	748	638
		2004/05	2006	2007	2008	2009	2010
*Woman b) 55 U/L	Value%	16%	11%	17%	18%	13%	12%
	N	459	647	601	505	770	701

*Reference values

Whereas alcohol consumption is extensive to exposed and non-exposed population, GGT enzyme values continue to be highest in exposed population, so, it is possible to minimize alcohol influence in increased serum concentration of this enzyme.

5. Liver infection morbidity (B and C Hepatitis) (2000- 2008)
Concerning B Hepatitis, reported cases in the studied population corresponded to all cases (100%) registered in the Alentejo Region in 6 of 9 studied years and, in the year 2000, highest case numbers was recorded (**Table 7**).

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Table 7: Hepatitis B casuistry in Beja and Évora between 2000 and 2008

	2000	2001	2002	2003	2004	2005	2006	2007	2008
B Hepatitis (Beja)	**4**	**1**	1	1	1	1	0	2	0
B Hepatitis (Évora)	**3**	0	2	1	2	0	2	0	**1**
B Hepatitis (Alentejo Region)	**7**	1	3	3	3	4	2	3	1
Beja and Évora casuistry (%) in Alentejo	**100%**	**100%**	**100%**	67%	**100%**	25%	**100%**	67%	**100%**

Concerning C Hepatitis, major recorded cases number was 44 cases in Évora, in 2001. The casuistry of those districts (Évora and Beja) on Alentejo Region, corresponded to all cases in 2 of 9 studied years (**Table 8**).

Table 8: Hepatitis C casuistry in Beja and Évora between 2000 and 2008

	2000	2001	2002	2003	2004	2005	2006	2007	2008
C Hepatitis (Beja)	2	1	0	1	0	0	10	6	0
C Hepatitis (Évora)	20	**44**	10	5	11	**2**	2	1	4
C Hepatitis (Alentejo Region)	22	47	10	8	18	5	15	11	6
Beja and Évora casuistry (%) in Alentejo	**100%**	96%	**100%**	75%	61%	40%	80%	64%	67%

Although liver affectation associated with B and C Hepatitis virus infection can evolve to chronic hepatitis or liver cancer (Padua, 2009), it was not possible to compare infected exposed and non-exposed people because it was considered classified information. This fact didn't allow to evaluate the liver virus infection influence in liver cancer diagnosis in exposed and non-exposed people as it was suggested by Fleming et al., (2002) e Svircev et al., (2009).

6. Liver cancer incidence in exposed and non-exposed population

Excepting 2002 and 2006, assessment of incidence liver cancer in studying period allow us to refer that exposed population presents more cases when compared to non-exposed population, (**Figure5**).

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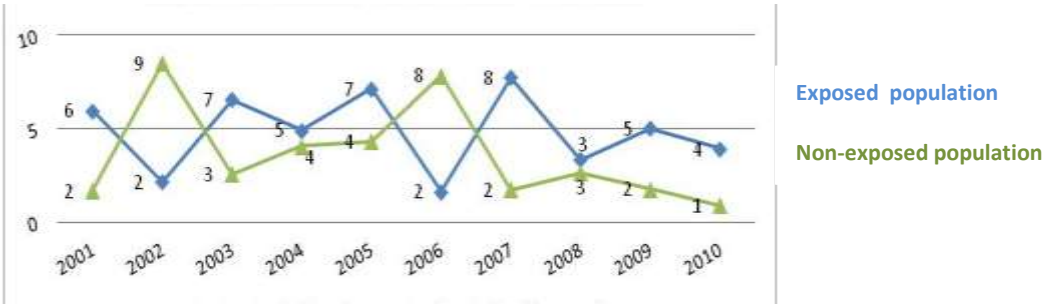

Figure 5: Liver cancer incidence rate evolution /100.000 inhabitants

It is also possible to verify that percentage of cancer diagnosis was superior in the exposed population, compared with non-exposed population (**Figure 6**). In 2001, 2007, 2009 and 2010, this percentage exceeded 80%. This means that of all established cancer diagnoses, more than 80% were in cyanobacteria toxins exposed population.

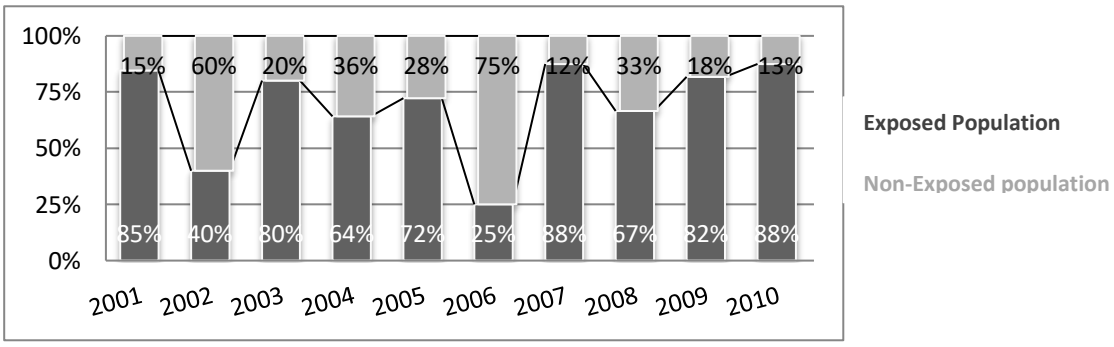

Figure 6: Percentage of liver cancer diagnosis in exposed and non-exposed population

It is important to refer that liver disease incidence could be also influenced by patients exportation or changes in tap water consumption habitudes but the water consumption pattern kept similar during the analysis period such as aged inhabitants number meaning longer cyan bacterial toxins exposition. This prolonged exposition was essential to indict causal relationship between cyan bacterial toxins and liver disease incidence (You, 1995; Svircev et al., 2009). Liver cancer incidence in this exposed population reached 85% of diagnosed according medical notification in epidemiological surveillance system context.

7. Relative Risk (RR) associated with the cyan bacterial toxins continuous exposition (2001-2010)
Relative Risk (association force) between exposition factor (cyanobacteria toxins) and liver cancer development, reached values over 1 in 8 of 10 studied years, meaning, according Mausner & Kramer (2007), that there is a positive association and potentially causing disease
Relative Risk presented highest value in 2007 and 2010 (4.4), evidencing that in those years, being exposed and get liver cancer risk was 4.4 times higher than getting liver cancer without exposition. This measure association was less expressive in 2004 (RR=1. 2) and 2008 (RR=1. 3) (**Table 9**).

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Table 9: Relative Risk associated with liver cancer between 2001 and 2010

	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Liver cancer	3,5	0,3	2,6	1,2	1,6	0,2	4,4	1,3	2,8	4,4

8. Attributable Risk (AR) associated with the cyanobacteria toxins continuous exposition (2001-2010)

Attributable Risk for liver cancer in population stood between 1% (2005) and 63%in 2007 and 2010 (**Table 10**). This means that in 2007 and 2010 63% of all appeared liver cancer cases in studied population may be assigned to cyanobacteria toxins exposition. In exposed population AR was 17% in 2004 and 77% in 2007 and 2010. That means if we could remove exposition factor in the exposed population, 77% of liver cancer cases could be avoided in the exposed population in 2007 and 2010.

Table 10: Attributable Risk in population and exposed population between 2001 and 2010

	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Attributable risk in population	53%	RR≤1	36%	RR≤1	1%	RR≤1	63%	RR≤1	42%	63%
Attributable risk in exposed population	72%	RR≤1	39%	17%	39%	RR≤1	77%	20%	64%	77%

Relative Risk value (4.4) in 2007 and 2010 surpassed Odds ratio value (2.46) revealed by Yu, Zhao, & Zi, (2001), indicting an association between continuous cyanobacterial toxins exposition and oncological liver disease in Portugal such as described in China. This result also meets those results disclosed by Fleming et al. (2002)

Final considerations

This ecological study allows to confirm the eutrophic condition of considered reservoirs based on Chlorophyll-*a* and Phosphorus recorded values.

Phytoplankton characterization through laboratory identification allows for drafting tables where cyanobacteria responsible for expressive blooms in studying reservoirs were entered, in particular non Nitrogen fixing genders (*Microcystis*e *Oscillatoria*) and Nitrogen fixing genders (*Anabaena* e *Aphanizomenon*). All of them produce liver toxins that promotes liver disease by continuous exposition.

During selected period those reservoirs were used to produce drinkable water in Beja and Évora districts, belonging to Alentejo Region. People supplied with this water was selected as exposed population and people not supplied was selected as non-exposed population.

Both districts presented in studying period an aging population and comparing water consumer indices *per capita* allow complete for highest water consumption in the exposed population since the beginning of considered period. Laboratory data confirm that exposed population presented highest indicators of liver condition (liver enzymes AST, ALT, GGT), and this circumstance can report a liver injury, associated with an absent factor in non-exposed population.

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Excepting 2002 and 2006, liver cancer incidence was highest in exposed population and after relative risk evaluation, it was possible to conclude that chances of being exposed and get liver cancer were generally higher than not being exposed and get liver cancer, indicating the presence of a positive and consistent causality relationship.

The attributable risk analysis allows to understand that some studied years presented high percentage of liver cancer cases in exposed population and those cases may be assigned to cyanobacterial toxins exposition.

This study reaffirmed in Alentejo an association already indicated in international studies, between liver disease (liver cancer) and systematic cyanobacterial toxins exposition trough drinkable water.

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