EVALUATION OF THE EFFECTS OF FLUORIDE AND ASSOCIATED WITH LEAD IN ANIMAL MODEL AND PHYSICAL-CHEMICAL ANALYSIS OF PUBLIC SUPPLY WATER AND OF THE SINOS RIVER IN THE SOUTH OF BRAZIL

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ABSTRACT

Fluoride related to caries prevention is at the center of a scientific controversy. Studies show that fluoride causes damage to health and the environment, as well as reducing IQ in children. The fluoridation of drinking water, mandatory in Brazil, has repercussions over the whole society. One of our objectives was to know the concentrations of fluoride (F) and toxic metals of Sinos River, treated water and final consumers of the cities of Campo Bom (CB), Novo Hamburgo (NH) and São Leopoldo (SL), as well as the groundwater from Ivoti, located in southern Brazil. We also evaluated in rats the effects of F and, in association with lead (Pb), on thyroid hormones and the Total Antioxidant Capacity (TAC). Three groups of rats were exposed to different waters: G1-Control with distilled water (DW); G2-DW with 25ppm (F); G3-DW with 25ppm (F) + 30 ppm (Pb). The Sinos River has an average concentration of 0.0735 mg.L^{-1} of F. But the F of both the water treated by the ETA of SL as well as in the final consumers of SL had concentrations above 0.9 mg.L^{-1} (State Ordinance No. 10/1999). In addition, we verified the presence of Pb and Cr (VI) in all types of water. The results with the animals showed a significant difference in T3 (p=0.032) and in T4 (p=0.043) from G3 to G1. In TAC, the difference was significant from G2 to G1 and G3 (p=0.007), showing that F and F with Pb interfere with the endocrine and antioxidant functions of rats. In addition, the fact that there are water fluoridation failures shows that the population is exposed to health risks. We confirm that drinking water fluoridation needs to be demystified and reconsidered as a public health intervention.

Keywords: Fluoride, Water Fluoridation, Lead, Toxic Metals, Thyroid, Antioxidant Capacity.

1. Introduction

The fluoridation of water has been considered essentially positive by society. However, fluoride and other emerging pollutants like toxic metals that are being intensified by human action, are a global concern in conflict with the provision of safe water to the population (UNESCO 2009; WHO 2016) giving rise to a more rational analysis of the benefits and costs of drinking water fluoridation (Mullenix 2014; Peckham and Awofeso 2014; Ko and Thiessen 2015; Hirzy et al. 2016).

Fluoride (F) is an halogen and a highly reactive element (Jasinski 2016). The phosphate industry is the basis for the manufacture of fertilizers, aluminum extraction and phosphorous (Denzinger et al. 1979), which is a process that generates, among other pollutants, an immense amount of toxic fluoride residues (Schmidt et al. 1995; Loureiro et al. 2008) that are converted into by-products for disposal or commercialization for use in water fluoridation (Glasser 1998; Bryson 2004). Environmental tragedies triggered the study of fluoride toxicity (Roholm 1937; 1937a). The recommendation of the World Health Organization (WHO) is a maximum fluoride value of 1.5mg.L^{-1} (ppm) in drinking water (Fawell et al. 2006). The maximum contaminant level (MCL) and its target (MCLG) for fluoride in drinking water is 4 mg.L^{-1} (or ppm), but experts suggest that if there is evidence of a chemical causing cancer the MCLG is set at zero as is for lead (Thiessen 2013). According Spittle (2014) the only safe level of fluoride in drinking water is zero.

The impacts of fluoride on health and its interference with the metabolism have been the subject of extensive investigations related to reduced thyroid function, oxidative stress, early aging, osteosarcoma, as well as a higher incidence of bone ruptures, arthritis, mutagenicity and other pathogenic effects (Waldbott 1980; Yiamouyiannis 1983; Shivarajashankara et al. 2002; Bassin et al. 2006; Ozsvath 2008; Kharb et al. 2012; Ravula et al. 2012; Pain 2017; PHE 2018). Dental fluorosis is chronic fluoride toxicity (Denbesten and Li 2012) and is already a public health problem in several countries such as in USA (Lewis and Banting 1994), India (Susheela et al. 2005; Reddy 2009) and in Brazil (Cangussu et al. 2002; Domingos 2009; Komati and Figueiredo 2013).

Fluoride is a neurotoxic element (Grandjean and Landrigan 2014). It is cumulative in the pineal gland (Mullenix et al. 1995; Luke 2001) and causes adverse effects on neurotransmitters, impairs mental functions and reduces children’s intelligence (Trivedi et al. 2007; Valdez-Jiménez et al. 2011; Choi et al. 2012; Saxena et al. 2012; Vandenberg et al. 2012; Peckham et al. 2015, Bashash et al. 2018). Studies associating fluoride with IQ reduction in children (Rocha-Amador et al. 2007; Tang et al. 2008; Hirzy et al. 2016; Bashash et al. 2017) show that they are among the minorities most vulnerable to fluoride and lead damage and neurotoxicity, as well as athletes, diabetics, and the kidney deficient (Marcus 1986; White 2004; Wimalawansa 2016; Wasana et al. 2017). These effects are aggravated in the presence of lead which fluoridation may intensify with corrosion in the pipes (Masters et al. 2000; Coplan et al. 2007; Maas et al. 2007; Hirzy et al. 2014).

Fluoride is considered an endocrine disruptor (Waissmann 2002; NRC 2006; Vandenberg et al. 2012; Jaishankar et al. 2014; Pain 2017). Its toxic effects significantly impact antioxidant defense systems (Rzeuski et al. 1998; Ruiz-Payan 2006; Shivarajashankara et al. 2002; Shivarajashankara and Shivashankara 2012). Combined with other metals such as lead, fluoride increases the production of free radicals in the brain (Chinoy and Shah 2004) and its synergistic effects on humans is a critical factor for public health (Stackelberg et al. 2015; Wasana et al. 2017).

The fact is that rates of dental cavities have declined in most of the developed nations in the last 40 years due to the increase of fluoride intake through food and toothpaste (Yiamouyiannis 1990; Colquhoun 1993; Levy et al. 2003; Jones et al. 2004; Fagin 2008) making it difficult to determine the amount consumed (Heller et al. 1997; Jha et al. 2011), thus requiring a review of fluoridation parameters (Levy and Guha-Chowdhury 1999; Levy et al. 2003; Connett et al. 2010; Peckham and Awofeso 2014). Relevant discovery was that the beneficial effect of fluoride is topic (Warren; Levy 2003; Fagin 2008; Buzalaf et al. 2011; Peckham and Awofeso 2014).

In view of the relevance of this theme to the whole society, we evaluated in animal model the effects of water with fluoride and synergisms of fluoride with lead on thyroid hormones and on total antioxidant capacity (TAC). We also analyzed the physico-chemical parameters, including the fluoride and toxic metals of the Sinos River, of treated water and of end-user water, as well as the groundwater of cities in the Sinos Valley region in southern Brazil.

2. Materials and methods

2.1. Identification of the study area

The Sinos Valley region is located in the state of Rio Grande do Sul, Brazil, which includes the cities of Campo Bom (CB), Novo Hamburgo (NH) and São Leopoldo (SL) that capture the water of the Sinos River for the public supply and Ivoti, which uses groundwater. The Sinos River is the source of raw water for approximately 1.2 million inhabitants, covering 32 municipalities with an area of 3,820 km2 in its route about 190 km (FEPAM 2017). The Sinos River is heavily impacted by domestic and industrial sewage, mainly in the region of Novo Hamburgo due to the leather-footwear industries located in the area (Scalon et al. 2010).

2.2. Collection and analysis of water

2.2.1. Raw and treated water from Sinos River

The raw water of the Sinos River is the source for the abstraction of water from the cities of CB, NH and SL for the proper treatment and distribution by the ETAs to the population. The catchment points are shown in Figure 1, at the coordinates 29°41'28.3"S 51°02'35.6"W (CB); 29°43'54.7"S 51°05'01.9"W (NH); 29°45'37.6"S 51°08'08.6"W (SL). The collections of both raw and treated water, ready for distribution, were carried out in the respective ETAs. The three sites were sampled at two distinct time points covering one sampling in the winter and one sampling in the summer, with the first collection being performed on the winter morning on 21/08/2017 (9 a.m. at 9°C) and the second on the summer morning of 10/01/2018 (9 a.m.at 25°C).

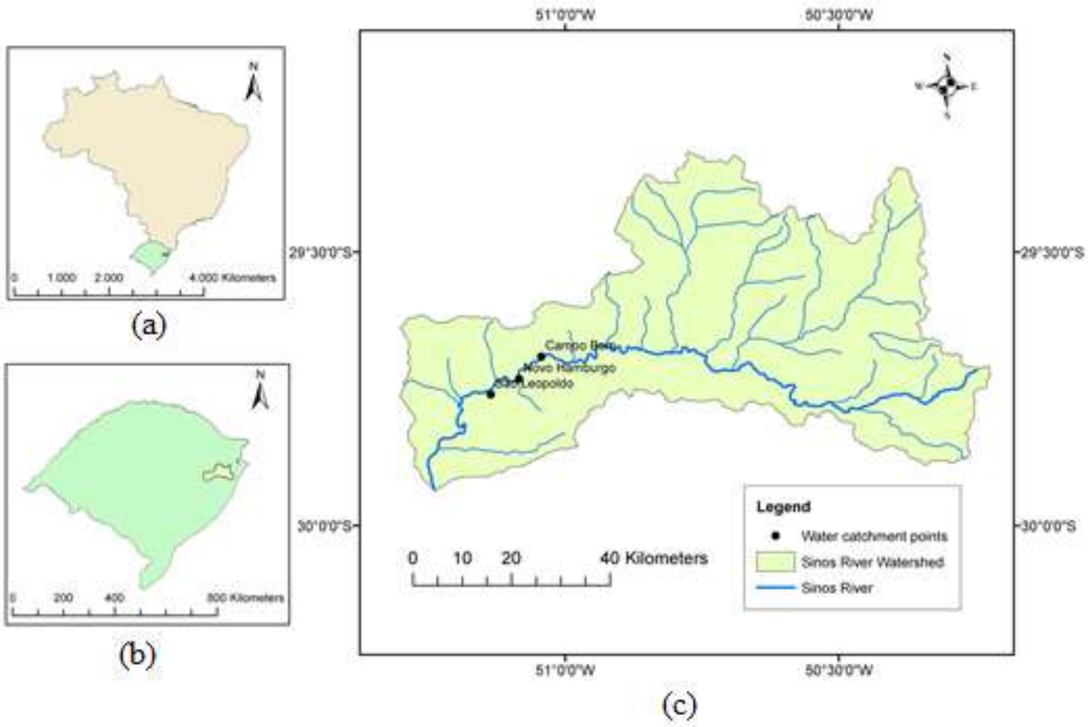


Fig. 1. (a) Map of Brazil with the State of Rio Grande do Sul (RS) in green. (b) Map of the RS state with the region of the Sinos River Bacin in yellow. (c) Map of the Sinos River Watershed with the identification of the three water catchment points (Campo Bom, Novo Hamburgo and São Leopoldo).

The three concessionaires that collect water from the Sinos River in the cities of CB, NH and SL follow the Brazilian legislation of the Ministry of Health (BRASIL 2011), Ordinance No. 5/2017, which regulates the drinking water standard for human consumption. Specifically for fluoride ion concentration in the State of Rio Grande do Sul, they follow the Ordinance No. 10/1999 of the Department of Health, which defines levels of fluoride ion concentration in water for human consumption. In the municipality of CB the concessionaire captures the raw water of the Sinos River that supplies the cities of Campo Bom, Estância Velha, Portão and Sapiranga, covering a population of 224,149 inhabitants (COREDE 2015). The treatment of the water carried out in the CB ETA follows the conventional model. It uses aluminum sulfate coagulant, chlorine disinfectant and fluosilicic acid for fluoridation. The municipality of NH has a population of 244,007 inhabitants (COREDE 2015). The NH ETA uses as the main coagulant/flocculant agent a product called aluminum polychloride and as an extra coagulant/flocculant an organic product based on tannin and chlorine-based products as a disinfecting agent. To fluoride the water, it uses flu silicic acid and sodium fluosilicate. The municipality of SL has 226,546 inhabitants (COREDE 2015) and the concessionaire responsible for the distribution of SL drinking water captures the raw water of the Sinos River in the two treatment units, using sodium hypochlorite in the disinfection process and fluosilicic acid for fluoridation.

2.2.2. Ivoti Groundwater

Ivoti groundwater was collected in three artesian wells, from the well SB3 on 21/08/2017, and from SB15 and SB50 on 10/01/2018. Water from the Ivoti wells was collected after treatment with sodium hypochlorite only at the site of collection and before being distributed to the population. For this reason, it was considered as treated water in the statistical analysis of the results. The municipality responsible for capturing and distributing water to the population of Ivoti follows Brazilian legislation relevant to drinking water (BRASIL 2011), but does not artificially fluorinate the water.

2.2.3. End-user water

Three collections of water samples were made from end-users on 11/05/2017, 08/21/2017 and 10/01/2018, from 2 sites (1 Hospital and 1 School) in each of the cities (CB/NH/SL). There were 18 end-user water samples in total.

2.3. Physical-chemical parameters analyzed

The physico-chemical parameters of the analyzed waters were fluoride, lead, total chromium, hexavalent chromium, iron, manganese, mercury, nickel, nitrates, nitrites, sodium, zinc, pH, total solids and turbidity. All analyzes to determine the physicochemical parameters in the water samples were performed by the Laboratory of the Analytical Center of the Feevale University, according to the Standard Methods for the Examination of Water and Wastewater of the American Public Health Association (APHA) of 2017.

2.4. Animal model

Fifteen (15) male Wistar rats were used from the Feevale University Animal Hospital, with approximately 40 days and mean weight of 163 g. They were exposed to a light/dark cycle of 12 hours, in an air-conditioned environment (22 ± 1°C and 50 ± 10% RH), with standard feed of industrialized ration and water *ad libitum*. All the cages had regular exchanges of the drinkers, being then measured the amount of water ingested by cage. The fifteen rats were randomly divided into three groups of five rats for 45 days of treatment, distributed as follows: Group 1 (G1) Control, composed of 5 rats, supplied with distilled water; Group 2 (G2) Fluoride, composed of 5 rats exposed to water with 25 mg.L-1 of fluoride (fluosilicic acid [H2SiF6]), Group 3 (G3) Fluoride + Lead, composed of 5 rats exposed to water with 25 $mg.L^{-1}$ of fluoride (fluosilicic acid [H2SiF6]) added to 30 $mg.L^{-1}$ of lead acetate $[Pb(C_2H_3O_2)_2]$ (Sawan et al. 2010; Fioresi 2011). The concentration of 25 $mg.L^{-1}$ of fluoride was adopted by reproducing in rats the same effects as 2 $mg.L^{-1}$ of fluoride detected in human plasma (Sawan et al. 2010), and it's near the concentration we found in final consumers water. As the distilled water together with the used agents was slightly acidic, the pH of the water was adjusted to around 7. The water ingested in each group was changed and measured at intervals of 2 to 3 days and the weight of the animals was recorded once a week. Minutes before the examination, the rats were anesthetized by the use of inhalant pharmacological agents (Isoflurane) (Paiva et al. 2005). The rats were then decapitated, blood was collected and aliquoted into metal free tubes, stored in the refrigerator until analysis for quantification of serum levels of TSH, T3 and T4 and TAC. The protocol for the use of animals in this experiment was approved by the Animal Use Ethics Committee (CEUA) of the Feevale University under the number 02.17.061.

2.4.1. Biochemical analyzes of serum levels of T3, T4 and TAC

The serum levels of thyroid hormones T3 and T4 total and free in the blood of the rats were determined by the immunoassay test in the chemiluminescent equipment (ECiQ VITROS). The biochemical analyzes were performed by the Biomedicine Laboratory of Feevale.

For the determination of TAC in the blood of the rats, the FRAP (Ferric Reducing Antioxidant Power) method was used according to Benzie and Strain (1996; 1999) characterized by the reduction of iron (Fe^{3+} in Fe^{2+}) based on the electron transfer reaction of the phenols. The analyzes were carried out at the Pharmacy Laboratory of the University of Feevale.

2.5. Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for Social Sciences v.24.0 (SPSS) software. For the water analysis the values obtained did not present a normal distribution and were analyzed by the Kolmogorov-Smirnov test. The Kruskal-Wallis non-parametric test was used for the analysis of medians, means and standard deviation, and correlations between data for when the *p* value was significant ($p < 0.05$). The Kruskal-Wallis non-parametric test followed by Dunnett was used for the analysis of the hormones. For the statistical analysis of the weight of the rats ANOVA was used, followed by Tuckey.

3. Results and discussion

3.1. Analysis of raw water from the Sinos River, treated water, groundwater and end-user water

The statistical analysis of the results of the physical-chemical parameters of the water samples does not present normal distribution (Kolmogorov-Smirnov test). The data of all types of water analyzed are shown by type of water: raw water; treated water (including groundwater); end-user water from the three cities (CB, NH, and SL), and are expressed by their medians, means and the standard deviation (Table 1).

Table 1. Physical-chemical parameters of raw, treated and end-user water from Campo Bom, Novo Hamburgo and São Leopoldo.

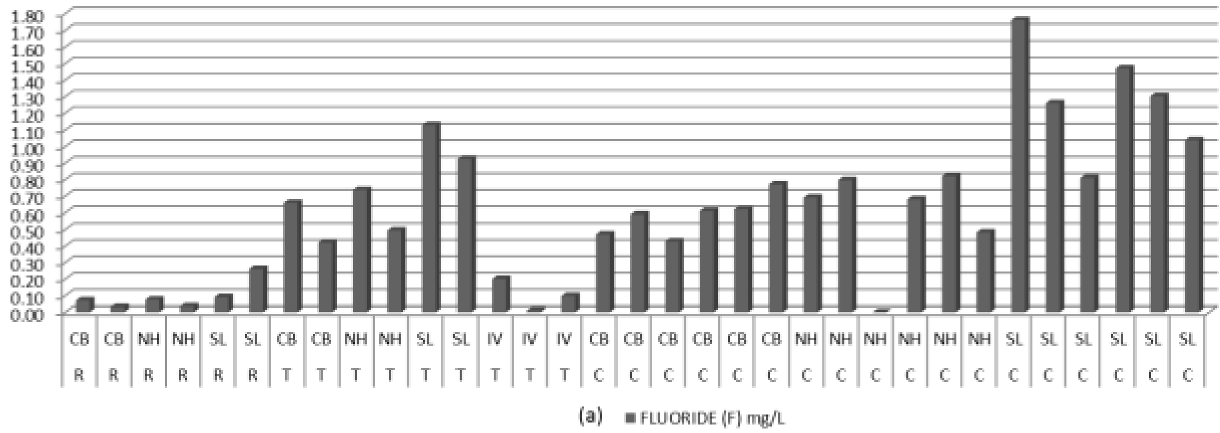
	Parameter	Fluoride	Lead	Chromium Total	Chromium VI	Nitrate	Nitrite	Iron	Manganese	Mercury	Nickel	Zinc	Sodium	pH	Total Solids	Turbidity
		$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$ N in NO_3	$mg.L^{-1}$ N in NO_2	$mg.L^{-1}$	$mg.L^{-1}$	$\mu g .L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$	$mg.L^{-1}$		$mg.L^{-1}$	NTU
RAW WATER	Median	0.074	0.009	0.015	0.001	0.668	0.050	0.870	0.104	0.020	0.031	0.005	6.100	6.91	120.5	27.55
	Mean	0.094	0.014	0.051	0.012	19.315	0.050	2.600	0.287	0.020	0.031	0.028	6.425	6.93	153.3	49.20
	S.D.	0.085	0.113	0.065	0.026	45.75	0.025	4.621	0.493	0.000	0.001	0.045	0.814	0.18	91.0	64.96
TREATED WATER	Median	0.494	0.005	0.015	0.007	0.961	0.001	0.030	0.022	0.020	0.030	0.005	9.400	6.96	115.0	0.20
	Mean	0.519	0.005	0.015	0.005	5.051	0.001	0.037	0.328	0.026	0.031	0.025	18.956	6.88	157.2	0.30
	S.D.	0.380	0.001	0.001	0.004	6.742	0.000	0.020	0.028	0.018	0.001	0.030	21.200	0.75	78.7	0.28
CONSUMERS WATER	Median	0.730	0.006	0.017	0.004	0.687	0.001	0.030	0.022	0.020	0.032	0.020	9.330	6.57	101.0	0.60
	Mean	0.811	0.006	0.090	0.004	0.818	0.002	0.034	0.312	0.032	0.031	0.023	9.470	6.52	108.0	0.72
	S.D.	0.421	0.001	0.109	0.003	0.353	0.003	0.013	0.022	0.037	0.001	0.020	2.705	0.75	45.3	0.53

The table 1 presents the results of each parameter whose name and unit of measure are described in the title of each column.

The results are expressed by medians, means and standard deviation (S.D.) by water type:

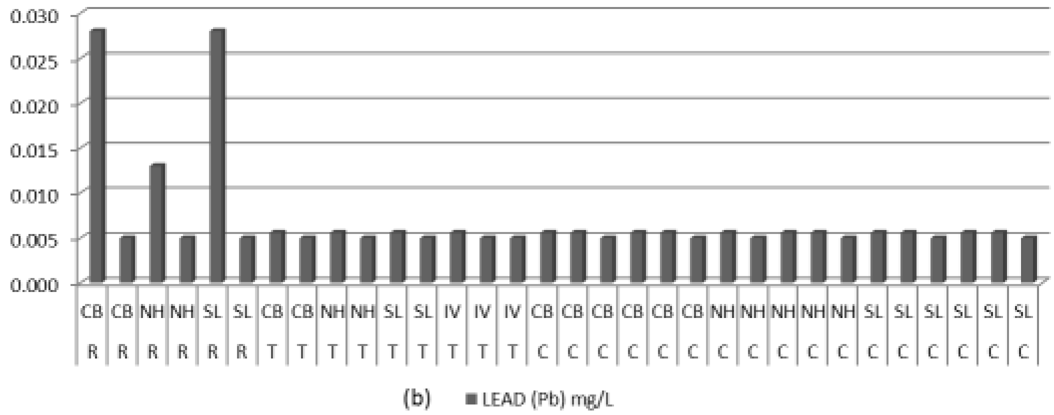
- Raw Water (n = 6); - Treated Water (n = 9); - Final Consumers Water (n = 18).

The present study emphasizes fluoride and its synergistic action with lead, therefore, the results of all analyzes of these two parameters are shown by water type and city and are presented in Figure 2a for fluoride (F) and Figure 2b for lead (Pb).



The results of fluoride (F) concentrations are expressed em mg.L^{-1} (ppm) by water type: Raw Water (R) n=6; Treated Water (T) n=9; and Final Consumers Water (C) n=18, and City (CB, NH, SL). The dates of the respective results are described in the methodology (topic 2.2).

Fig. 2a. Physicochemical analysis of Fluoride of raw, treated and end-user water.



The results of lead (Pb) are expressed em mg.L^{-1} (ppm) by water type: Raw Water (R) n = 6; Treated Water (T) n = 9; and Final Consumers Water (C) n = 18, and City (CB, NH, SL). The dates of the respective results are described in the methodology (topic 2.2).

Fig. 2b. Physicochemical analysis of Lead (b) of raw, treated and end-user water.

According to Brazilian legislation, the amount of fluoride in the water supplied to the population must be at most 1.5 mg.L^{-1} (BRASIL 2011). However, the Maximum Allowed Value (VMP) of the fluoride applied in the state of Rio Grande do Sul, follows the State Ordinance no. 10/1999 which defines the concentration of fluoride between 0.6-0.9 mg.L^{-1} in end-user water (Portaria 1999). In fact, in April 2015, the US Department of Health and Human Services lowered the recommended amount of fluoride in drinking water to 0.7 mg.L^{-1} nationwide. This change was the first since 1962, when the federal government suggested an increase to 1.2 mg.L^{-1} in areas with colder climates and 0.7 mg.L^{-1} in warmer areas where people consume more water (HHS 2011).

The water fluoride treated by the ETA from SL presented concentrations of 1.1285 and 0.9245 mg.L^{-1} respectively (Fig. 2) in the two collections, which exceeded the MPV of 0.9 mg.L^{-1} of State

Ordinance 10/99 (Portaria 1999). As the distribution networks are exempt from submitting fluoride analyzes to the community (Portaria 1999), we can assume that the fluoridation carried out in the water treatment process is flawed, apart from the worrying fact that it is not feasible to monitor the excess of fluoride in drinking water by users. The treated water of NH and CB presented all values within the standard of Ordinance 10/99. Another study by Lacerda (2016) found fluoride at a mean of 0.541 $mg.L^{-1}$ in the treated NH water.

The water fluoride of the end-users of SL reflected the result of the treated water of that city (Fig. 2), being above the MPV of 0.9 $mg.L^{-1}$ (Portaria 1999) in 83.3% of the six samples (Hospital-SL: 1.760 / 1.2595 / 0.8102 $mg.L^{-1}$; School-SL: 1.470 / 1.3014 / 1.0378 $mg.L^{-1}$).

Ivoti's groundwater presents relatively low fluoride levels, with a mean of 0.102 $mg.L^{-1}$ (Figure 2a). Groundwater is the source of drinking water for many communities in many parts of the world where high concentrations of fluoride are found above the WHO limit of 1.5 $mg.L^{-1}$ (Edmunds and Smedley 2001). In rural areas of South Africa, groundwater is the only source of water and elevated levels of fluoride are causing a preponderance of dental fluorosis and negative impacts on human health and the environment (Odiyo and Makungo 2012). The central depression region of RS/Brazil has a high concentration of fluoride in the groundwater, and the study carried out in the city of Santa Maria, located in that region, fluoride contents in most wells presented high values of fluoride, between 0.5 up to 3.3 mg $.L^{-1}$ (Terra 2015).

In relation to raw water, in Brazil, we can compare the results found and presented in Table 1 with the CONAMA Resolution 357/2005 that establishes maximum values (VM) in water bodies in the national territory for the management of the uses of this resource (CONAMA 2005).

The Sinos River raw water had a mean fluoride concentration of 0.094 $mg.L^{-1}$ (Table 1). Little is known about this parameter because the ETAs perform this analysis after the water fluoridation. One of the few studies that analyzed fluoride from the Sinos River raw water in Novo Hamburgo was conducted by Lacerda (2016) and found lower values at a mean of 0.036 $mg.L^{-1}$ (S.D. 0.033).

The lead in the Rio dos Sinos water presented a mean of 0.0140 $mg.L^{-1}$ (SD 0.113) and was found in all points of the first collection (Fig. 2b) with values close to the MPV of Class 3 of the Brazilian legislation CONAMA 357/2005 (0.033 $mg.L^{-1}$) (CONAMA, 2005). This fact was determinant for the use of lead in the animal model. Robaina et al. (2002) also found lead in high levels of contamination in the Sinos River. Oliveira (2015) found 0.145 $mg.L^{-1}$ of lead in the lower section of the Sinos River in two collections conducted in 11/2011, and the general mean of 0.039 $mg.L^{-1}$, presenting the river classification as 50% of the periods classified as Class 3 and 33% as Class 4. Lacerda (2016) found lead in raw water at a mean of 0.014 $mg.L^{-1}$ (SD 0.026) and treated on average 0.010 $mg.L^{-1}$ (SD 0.019), indicating that there were samples with lead above these limits. Costa et al. (2014) found high levels of lead (0.023 $mg.L^{-1}$) in the Sinos River in Campo Bom, in the summer, and included the source and mouth of the Schmidt Stream, which flows into the Sinos River. Toxic contamination was considered high due to the presence of heavy metals cadmium and lead.

Total chromium presented a mean in the raw water of 0.051 $mg.L^{-1}$ (S.D. 0.065) and in the end-user water of 0.090 $mg.L^{-1}$ (S.D. 0.109). The presence of chromium of 0.04 $mg.L^{-1}$ was also verified in the Sinos River by Scalon et al. (2010). In the first collection, performed only in end-user sites, chromium appeared in all samples between a minimum of 0.187 $mg.L^{-1}$ (NH, Hospital) and a maximum of 0.287 $mg.L^{-1}$ (SL, School), well above the VMP. The legal maximum limits for chromium are both for raw and potable water, 0.05 $mg.L^{-1}$ (CONAMA 2005; BRASIL 2011). However, Cr VI was included in the subsequent analyzes and was actually detected in all treated water samples with a mean of 0.0053 $mg.L^{-1}$ (SD 0.004) and 0.004 $mg.L^{-1}$ (SD 0.003) in end-user samples. In raw water, Cr (VI) was 0.012 $mg.L^{-1}$ (S.D. 0.026), and the most significant sample in CB in the summer was 0.0660 $mg.L^{-1}$. Cr VI is a known carcinogen (EWG, 2011) and is responsible for the toxicity observed by the tannin effluents in the Vale do Sinos region (Lacerda 2016). Other studies have shown high levels of Cr VI contamination (Robaina et al. 2002; Costa and Klein 2006; Nascimento and Naime 2009; Nudler et al. 2009). Another study carried out in the Sinos River in 2011 found the presence of Cr (VI) in an average of 0.045 $mg.L^{-1}$ in Novo Hamburgo and 0.065 $mg.L^{-1}$ in São Leopoldo (Oliveira et al. 2012). Studies of rivers contaminated with chromium are important for the evaluation of aquatic ecosystems and the contamination of the environment, considering other synergistic or antagonistic interactions among the chemical products (Lambolez et al. 1994; Scalon et al. 2010).

Manganese presented an average of 0.287 mg.L-1 (SD 0.493) in Sinos River water, which is within the range of 0.1 $mg.L^{-1}$ for Class 1 and 0.5 $mg.L^{-1}$ for Class 3 permitted by CONAMA Resolution 357/2005 for fresh water (CONAMA 2005). However, in one of the CB samples a concentration of 1,291 $mg.L^{-1}$ was found, well above these permitted levels. Other studies carried out in the Sinos River also verified the presence of manganese, presenting an average of 0,104 $mg.L^{-1}$ (SD 0,054) (Lacerda 2016) and, at the Campo Bom catchment point, the river can be classified as a class 3 in 40% of the manganese analyzes (Nascimento et al. 2015). Manganese oxides are the only inorganic oxidants found in the environment that cause the rapid oxidation of Cr (III) to Cr (VI) (RAI et al. 1989). At least one hypothesis indicates that natural fluoride forms a soluble complex with minerals containing chromium (III) after which the dissolved Cr (III) comes into contact with the manganese dioxide (MnO2) and material contained in the aquifer, causing oxidation to Cr (VI) (Jacobs and Testa 2004). This indicates the complex synergistic effects that may occur in water among different pollutants, toxic metals and fluoride.

The table 2 shows the medians, means and standard deviation of the parameters that presented a significant difference ($p < 0.05$) in the comparative test between the mean values of the raw and treated water parameters.

Table 2. Comparison of physical-chemical parameters between raw and treated water.

	Parameter	**Fluoride** $mg.L^{-1}$	**Nitrite** $mg.L^{-1}$ N in NO_2	**Manganese** $mg.L^{-1}$	**Sodium** $mg.L^{-1}$	**Turbidity** NTU
RAW WATER	Median	0.074	0.050	0.104	6.100	27.55
	Mean	0.094	0.050	0.287	6.425	49.20
	S.D.	0.085	0.025	0.493	0.814	64.96
TREATED WATER	Median	0.494	0.001	0.022	9.400	0.20
	Mean	0.519	0.001	0.328	18.956	0.30
	S.D.	0.380	0.000	0.028	21.200	0.28
	p-Value	**0.026**	**0.008**	**0.003**	**0.026**	**0.0001**

The table 2 presents the results of the parameters that obtained p values ($p < 0.05$) using the Mann-Whitney test, whose name and unit of measure are described in the title of each column. Data are expressed as median, mean and standard deviation (S.D.).

We emphasize that the significant difference in the increase of fluoride is the result of the process of intentional addition of fluoride in the drinking water by the concessionaires for compliance with the federal legislation (BRASIL 1974). The other parameters with significant difference, such as nitrite, manganese, and turbidity, which decreased, also result from the water treatment process, denoting its efficacy. However, the presence of these elements in raw water highlights the worrisome conditions of contamination of the water bodies of the studied region, evidencing the need for a better management and monitoring of the water resource in this region. A study by Blume et al. 2010 carried out in four sites in the Sinos River basin in 2007 and 2008, identified that the Sinos River was not suitable for the collection of water for human consumption in accordance with the Brazilian legislation CONAMA (2011). The results of another study in the Sinos River basin, including catchment points in 5 cities (including Campo Bom) suggested that the main pollutant of water was domestic sewage, with no metals and pesticides (Nascimento et al. 2015). On the other hand, Robaina et al. (2002) found lead, chromium and nickel in stream sediments that flow into the Sinos River. The increase of sodium from raw water to treated water raises the idea of treating the "hardness" of the water caused by the presence of calcium and magnesium with the use of sodium. In water treatment and filtration systems, softeners that normally use sodium or potassium ions are used to replace calcium and magnesium ions that create hardness in water (EPA 2005).

The comparison between treated and final consumer water shown in Table 3 showed a significant difference ($p < 0.01$) in total chromium and turbidity.

Table 3. Comparison between treated and end-user water.

	Parameter	**Chromium Total**	**Turbidity**
		$mg.L^{-1}$	NTU
TREATED WATER	Median	0.015	0.20
	Mean	0.015	0.30
	S.D.	0.001	0.28
CONSUMERS WATER	Median	0.017	0.60
	Mean	0.090	0.72
	S.D.	0.109	0.53
	p-Value	**0.000**	**0.002**

The table 3 presents the results of the parameters that obtained p values ($p < 0.01$) using the Mann-Whitney test, whose name and unit of measure are described in the title of each column. Data are expressed as median, mean and standard deviation (S.D.).

The lack of regulation for hexavalent chromium (Cr VI), the most toxic form, assumes that the total chromium measurement is 100% Cr VI in order to ensure that the greatest potential risk is addressed (EPA, 2016). The presence of chromium and especially chromium (VI) in treated and end-user water is problematic, mainly from raw water (table 1). Although the significant difference (p=0.000) in the reduction of chromium from the treated water to the end user is positive, this fact may be due to Cr (VI) reduction processes in this path. Another study carried out in the Sinos River by Oliveira et al. (2012) also found high levels of Cr (VI) in NH and SL. The Environmental Working Group (EWG 2011) released an analysis of more than 60,000 faucet water tests conducted nationwide, finding that hexavalent chromium is in the tap water of 31 of the 35 US cities surveyed, the highest levels being high in Norman, Oklahoma (12.9 ppb); Honolulu, Hawaii (2 ppb); and Riverside, California (1.69 ppb). In groundwater, one of the sources of Cr (VI) comes from the proximity of coal ash ponds, contaminating potable water wells as shown by Vengosh et al. (2016). It is known that the reduction of Cr (VI) to Cr (III) results in the formation of reactive intermediates that contribute to cytotoxicity, genotoxicity and carcinogenicity through cellular events and oxidative damage such as apoptosis, DNA mutations, chromosomal damage and oxidation of proteins and enzymes (Caprara et al. 2016; Taju et al. 2017).

The increased turbidity may be related to contamination processes originating from both the distribution network and the storage and distribution in the end users. The level of turbidity in the delivery systems may vary depending on the source of supply; the type of treatment; the operating conditions (e.g. pressure fluctuations and continuous or intermittent supply); and the characteristics, condition, complexity and integrity of the distribution network (WHO 2017). Disruption of the water supply may also lead to unhealthy material entering the pipelines and, due to back suction through leaks, damage and failure of the supply lines, it can be transported to end users when the supply is resumed (Khadse et al. 2016). In a study conducted in the Sinos River to identify the most relevant parameters contributing to seasonal variations in water quality using integrated statistical techniques, turbidity was one of the most significant parameters in the spring, demonstrating that this parameter can be used as an indicator for water quality assessment (Alves et al. 2018). Turbidity is an important parameter to be included in water safety plans to support water quality management (WHO 2017).

The other physical-chemical parameters analyzed presented values within the standard set by the legislation for raw water (CONAMA 2005) and proper for human consumption (BRASIL 2011).

The observation of some of the parameters analyzed outside the legal standard (e.g. fluoride, lead, chromium, hexavalent chromium and manganese) endorses the need for periodic analysis and studies that allow a monitoring and control of possible distortions of toxic elements, both in water from the Sinos River and in treated water and end-user water.

3.2. Analyzes of the animal model

3.2.1. Evaluation of parameters of water consumption and weight of rats

During the 45-day period of the experiment, the water of the animals was changed three times a week and there were 17 changes in total. The total water consumption in the Control group (G1) was 8.2 liters and the average per animal was 1.63 liters; for the Fluoride Group (G2) the total consumption was 7.9 liters, with a mean of 1.59 liters per animal; and for the Fluoride + Lead group (G3) the total consumption was 7.1 liters, with an average of 1.42 liters per animal. According to the normality test of Kolmogorov-Smirnov, the data do not present normal distribution. The Kruskal-Wallis test did not present a significant difference between the groups (p=0.15).

The animals were weighed at the beginning of the experiment and weighed on average 166 grams. The individual evolution of the weight of the animals was accompanied with weekly weighing. The initial and final weight and mean weight gain per group is shown in Table 4.

Table 4. Weight gain per group of rats exposed to Fluoride (G2) and Fluoride + Lead (G3).

Group	Initial Mean Weight (g)	Final Mean Weight (g)	Weight gain (g)	Dif. to G1 (g)	Dif. to G1 (%)
G1: Control	162	333	171	-	-
G2: (F)	170	353	183	12	7
G3: (F) + (Pb)	166	369	203	32	19

The table 4 shows the Initial and Final Weight (Mean) and the Weight gain of each group of rats in the period of 45 days of exposure in the unit of measurement in grams (g). The weight gain of Group 2 and 3 in relation to the control group is represented in the last two columns by weight (g) and percentage (%) respectively.

The statistical analysis of the influence of different exposures of the animal model on the weight of the rats showed a significant difference ($p=0.037$) (ANOVA followed by Tuckey), between G1 (Control) and G3 (Fluoride + Lead). This indicates that lead may have been the cause of the weight difference. The risks of adverse health effects and the toxicity of lead are related to the total body content of lead, an understanding that was possible through lead kinetics and its interference in all metabolisms (Moreira Moreira, 2004). Lead is associated with health problems ranging from mental retardation in children to hypertension and renal insufficiency (Jadhav et al. 1995; Gomaa et al. 2002; Hu et al. 2006), accumulating in the thyroid and other glands interfering with reproductive functions (Moreira and Moreira 2004), passing through breast milk (WHO 2001) through the placenta, increasing the rate of abortions and malformations (Peres et al. 2001; Hu et al. 2006). Lead exposure is also associated with increased risk of delinquency and antisocial behavior (Needleman et al. 1996; Coplan et al. 2007). It is known that heavy metals, including lead, are endocrine disrupters and also confirmed as being associated with obesity, which may indicate, along with environmental factors, the growing prevalence of overweight people (Grun and Blumberg 2006). The study by Sun et al. (2017) demonstrated that chronic exposure to 0.05% lead (Pb) results in weight gain and insulin-specific resistance causing disorders are related to the metabolism.

3.2.2. Evaluation of T3 and T4 parameters and Total Antioxidant Capacity (TAC)

The results of the T3 and T4 hormone and TAC analyzes by group are shown in table 5. The Kruskal-Wallis non-parametric test showed that there is a significant difference between the groups for T3, T4, and TAC.

Table 5. Results of the analysis of the hormones T3 and T4 and TAC.

Group \ Parameter	**T3** (ng/dL)	**T4** (µg/dL)	**CAT** (µMol/L)
CONTROL (G1)	**83.00** (77.00 - 87.00)	**3.95** (3.480 - 4.045)	**159.00** (155.50 - 167.50)
FLUORIDE (G2)	**90.00** (73.00 - 92.50)	**4.12** (3.295 - 4.270)	**491.00**** (463.50 - 640.00)
FLUORIDE + LEAD (G3)	**92.00**** (90.50 - 95.50)	**4.36*** (4.125 - 4.615)	**130.00** (116.50 - 163.50)
p-Value	**0.032**	**0.043**	**0.007**

Table 5 shows the results of T3 and T4 hormone and Total Antioxidant Capacity (TAC) analyzes per group. The significant difference between the groups was performed by Dunnett's Post Hoc test, showing that:
* In both T3 and T4 the difference is significant between G1 and G3: T3 ($p = 0.032$) and T4 ($p = 0.043$).
** In TAC the difference was significant from G2 to G1 and from G2 to G3.

These results with significant increase in T3 and T4 hormones in both groups, G2 (F) and G3 (F + Pb), in relation to the control group (G1), the difference being for T3 ($p = 0.032$) and T4 ($p = 0.043$), are denoting that the association of fluoride with lead interferes more than fluoride alone in T3 and T4 hormones. Most of the studies, contrary to our results, relate fluoride to the reduction of thyroid hormones, causing hypothyroidism, but both, lead and fluoride are identified as being an endocrine disruptor (NRC 2006; Vandenberg et al. 2012; Pain 2017). T3 and T4 levels are regulated by the pituitary gland through TSH, an acronym for the synthesis of thyroid stimulating hormone, whose function is to induce the thyroid to produce the two hormones triidotironine (T3) and thyroxin (T4) that help control the metabolism of the body (Brownstein 2014).

The study of Nascimento (2010) on the effects of exogenous treatment of T4 on plasma levels of T3 and T4 found levels of T3 (55.60 ± 1.93 ng/dl) in the control group (treated with saline solution for 10 days) and T4 (7.87 ± 0.33 µg / dl). Compared with the control group (Table 5), our result is about 30% higher in T3 and half in T4. In the study by Mota et al. (2004) performed in adult Wistar rats, the analysis of the hormones before a transplantation was performed, finding the pre-surgery T3 values of 46.7 ± 6.6 ng/dL and T4 of 1.9 ± 0.4 µg/dL and comparing with our results, this values are much lower.

Peckham et al. (2015) found that in fully fluoridated area people are almost twice as likely to report high prevalence of hypothyroidism compared to non-fluoridated area. Some studies have found, for example, that subclinical hypothyroidism in pregnant women results in reduced IQ in the offspring (Haddow et al. 1999; Klein et al. 2001) and that adults with subclinical hypothyroidism have a significant increase in coronary heart disease (Rodondi 2010). Dysfunctions on thyroid hormones are associated with patients with panic, anxiety, stress and depression syndrome (Fishman et al. 1985; Nascimento 2010). In Delhi, India, from a group of 90 children with dental fluorosis living in areas with endemic fluoride, not iodine deficient, 54.4% had well-defined hormonal disorders (Susheela et al. 2005). Other studies in China and Russia corroborate the relation fluoride/hypothyroidism with changes in thyroid hormones, including reduced T3 and increased TSH, in populations exposed to high levels of fluoride in the workplace or in water (Bachinskii et al. 1985; Mikhailets et al. 1996). Zhao et al. (1998) found that high fluoride content produced goiters in rats. Another study by Bobek et al. (1976) found decreases in plasma T3 and T4, as well as a decrease in the free T4 index and an increase in T3 uptake, consistent with the groups treated with Guan et al. (1988), in which a fluoride intake of 30 $mg.L^{-1}$ (F) in water resulted in significant decreases in thyroid function, weight decrease and effects on the morphology of thyroid. Another study found an overall reduction in thyroid tone in tumor-bearing animals (Walker-256) (Monte et al. 2005).

Lead is associated with various endocrine disturbances causing testicular atrophy and reduced sperm quality and quantity (Moreira and Moreira, 2004) and causing a significant increase in the rate of abortion, stillbirth, prematurity, decreased postnatal growth and increase the rate of malformations (Peres et al. 2001). Contamination with lead caused by corrosion in water pipes has been studied under various fluoridation conditions (Masters et al. 2000; Coplan et al. 2007; Maas et al. 2007; Hirzy et al. 2014) and have also shown that fluoride present in water increases the concentrations of lead in the blood in children (Masters et al. 2000) and in calcified tissues in rats (Sawan et al. 2010). The effects of lead on thyroid hormones were analyzed by Singh et al. (2000), who studied 58 men who were occupationally exposed to lead, concluding that levels of lead in the blood ($\geq$ 50 $\mu g.dL^{-1}$) may increase the release of thyroid stimulating hormone (TSH) from the pituitary without any significant change in circulating levels of T3 and T4.

The significant change in TAC (p=0.007) in the present study from G2 (Fluoride) to G1 (Control) and from G2 (Fluoride) to G3 (Fluoride + Lead) shows that fluoride and lead interfere with the total antioxidant capacity of rats. The factors that influence the reduction of TAC are alcoholism, smoking and exposure to radiation, herbicides, carbon monoxide, carbon tetrachloride, lead, arsenic, mercury, cadmium, aluminum and other toxic elements (Ferrari 2012). Basha et al. (2011) observed that the fluoride-induced increase in lipid peroxidation and decreases in antioxidants were more pronounced with the second and third generations compared to the first generation of rats. Bahrami et al. (2016) found that Toxoplasma gondii-infected mice found much higher blood levels of TAC in the control group (uninfected) on the order of 874.1 ± 128.3 (mmol/L). Other studies have shown that exposure of humans to lead compounds results in changes in the activity of antioxidant enzymes and oxidation of lipids in the blood (Kasperczyk et al. 2005; ATSD 2007). Chronic exposure to lead weakens the antioxidant system causing cardiovascular diseases by promoting oxidative stress associated with hypertension, increased activation of the angiotensin converting enzyme, increased levels of lipid peroxidation and lower levels of nitric oxide and total antioxidant capacity (Dursun et al. 2005; Nunes et al. 2015). Varol et al. (2013) demonstrated that TAC plays an important role in patients with endemic fluorosis. Our results showed that fluoride significantly increased the antioxidant defenses of rats, and fluoride in combination with lead also interfered with TAC, but in an inverse way, that is, reducing these defenses to levels below the control group, suggesting that effect of fluoride may be being suppressed by the synergism of fluoride with lead. This agrees with other studies in which the exposure to lead was associated with hypertension, increased activation of the angiotensin-converting enzyme, increased levels of lipid peroxidation and lower levels of nitric oxide and total antioxidant capacity (Chen et al. 2000; Villeda-Hernández et al. 2001; Nehru and Kanwar 2004; Payal et al. 2009; Alghasham et al. 2011).

4. Conclusion

The physicochemical analyzes of water show levels of fluoride above the maximum value allowed in treated water and in end-user water of São Leopoldo when compared to the state legislation. The presence of other metals, including lead, manganese and hexavalent chromium (in all types of water) were also observed, evidencing the need and relevance of the monitoring of the Sinos River water parameters and the public supply system. Results from the animal model showed that lead may have caused weight differences in rats and that there was also a significant increase in T3, T4 and TAC between groups, showing the interference of fluoride with lead in the thyroid and TAC functions. It remains the indication and the urgent need for complementary studies with rats including the analysis of other tissues such as the brain, as well as the repercussion of fluoride on the psychological and behavioral aspect of the animals to evaluate and confirm the data that evidence the reduction in IQ. The fluoridation process is subject to failure and evidence was found on the adverse effects of fluoride and fluoride with lead in the endocrine system exposing part of the population to risks. The need for the practice of artificial water fluoridation needs to be reconsidered as a public intervention in health. In addition, the need for further studies was evidenced, so as to elucidate the effects of fluoride together with the mechanisms of action with other toxic metals in living beings and in the environment.

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