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Possible effects of metals on learning disabilities in school-age children

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Abstract

There are reports that children's exposure to toxic metals is related to learning. The oxidative stress is considered the main mechanism involved in the pathophysiology of some metals poisoning. This study evaluated the levels of some toxic and essential metals in blood and hair in school-age children with learning disabilities. Also, we evaluated biomarkers of oxidative stress and performed biochemical and hematological analyses. Presence of metals in drinking water were also analyzed. It was found high concentrations of blood lead (Pb) and aluminum (Al) in hair and drinking water of study group. Moreover it, was found selenium deficiency in blood and hair samples of the study group. Biomarkers of oxidative stress, such MDA and reactivation index of δ-aminolevulinate dehydratase (ALA-RE), were increased in study group when compared with control group. Thus, the oxidative damage is involved in toxicity of metals. Furthermore, more studies are necessary to confirm the sources of contamination for metals.

Keywords: Children; learning disabilities; metals; lipid peroxidation; oxidative stress.

Introduction

Cognitive development is the ability of children to act in the process of thinking and learning, including perceiving, interpreting, remembering certain information and evaluating ideas. Children with cognitive delays suffer from slow learning, especially in the basic skills of reading, writing and mathematics (Chen et al. 2007). The cognition is considered as the main predictor of the learning ability (Siqueira et al. 2011). Some consequences of poor school performance in the lives of children with learning disabilities, such as low grades, need of special support classes, repetition and the suffering caused by the difficulty of relationships may influence adult life. It is therefore important to invest in the early diagnosis of the problem (Pastura et al. 2005).

Environmental contamination by chemicals from industrial activities, mining and agricultural production has been an increased concern in recent years due to a possible relationship to neurological and behavioral disorders in children (Silva et al. 2005). Children are more vulnerable than adults to the toxic effects of chemicals. They drink more water and food and breathe more air per unit weight than adults, being more exposed to chemicals present in air and water (Landrigan et al. 1998; Lloyd-Smith and Sheffield-Brotherton 2008). Furthermore, habits such as taking the hand to the mouth, playing and moving close to the soil contribute to a greater exposure (Silva et al. 2005). Exposure to chemicals at critical stages of physical and cognitive development may have serious long-term consequences for health (Lloyd-Smith and Sheffield-Brotherton 2008).

There are reports that exposure to various metals is related to learning. The relationship between children's exposure to multiple toxic metals and cognitive and behavioral deficits is a major public health problem. There are many unanswered

questions about exposure to metals in children, including the exposure degree through air, soil, water and food. This exposure may occur through ingestion, inhalation and absorption (Kordas et al. 2010).

Biomonitoring of trace elements in biological samples in human is very important for occupational and environmental health (Heitland and Koster et al. 2006). The trace elements are essential components of biological structures, but in higher concentrations than those required for biological functions they can be toxic (Oga et al. 2008). Many nonessential elements are very ubiquitous in the environment and are easily detected in human body tissues and fluids. Also, monitoring the nutritional status of essential elements is important because they participate in numerous biochemical mechanisms and are required by an organism to maintain its normal physiological function (Rodrigues et al. 2008).

Evidence shows that exposure to lead (Pb) in children can impair neurocognitive deficits and behavioral development (Surkan et al. 2007; Costa de Almeida et al. 2010). Screening blood lead levels in children has been widely recommended by health authorities as an important means to identify children at risk, which allows early intervention through resources to reduce long-term deficits (Moodie et al. 2010). Moreover, aluminum (Al) may also cause delays in neurological development in children because it is a highly neurotoxic element (Yumoto et al. 2001).

In addition, oxidative stress is the main mechanism involved in the pathophysiology of lead and aluminum poisoning (Ahamed et al. 2007; Nayak et al. 2010) and the main indicator of oxidative damage is lipid peroxidation, which can be determined by plasmatic levels of the biomarker malondialdehyde (MDA). The δ-aminolevulinate dehydratase (ALA-D), an enzyme involved in heme biosynthesis, is

highly sensitive to lead and it has been used to evaluate lead-induced oxidative damage (Jin et al. 2006).

In this line, the present study evaluated the levels of some toxic and essential metals and others xenobiotics in school-age children who had learning disabilities, living in a rural zone of southern Brazil. Furthermore, we evaluated oxidative stress biomarkers, such as MDA and ALA-D activity and ALA-D reactivation, by comparing the study group with children without learning disabilities living in an urban area. In addition, analysis of drinking water was carried in homes of some children, to assess the possible source of exposure to some metals.

Materials and methods

Subjects

Twenty school-age children (8-14 years old) of a rural area of southern Brazil, in municipality of Agudo, RS, Brazil, who had learning disabilities or cognitive deficits (study group), according to survey conducted by the Reference Center in Occupational Health (CEREST) of state of Rio Grande do Sul were enrolled in this study. A control group of twenty children of an urban area of Santa Maria, RS, Brazil - who had no cognitive deficits or learning disabilities - also participated of the study for comparison of oxidative stress biomarkers with the study group, since there are no reference values for these biomarkers.

The responsibles have agreed to the participation of children in this study. The consent term was applied by medical of CEREST and this study was approved by the

committe of ethics of the Federal University of Santa Maria, Santa Maria, RS (CAAE 0147.0.243.000-06).

Cognitive assessment

The cognitive evaluation of the children of study group was conducted by psychologist of CEREST. To evaluate the learning problem was used the Bender Test for skill visomotor assessment and R2 Intelligence Test (nonverbal intelligence test for children) was used for intellectual assessment as well as interviews, home visits and observations of children's behavior. The Bender Test consists of nine figures that are presented separately for the children to copy them as best as they can in a blank sheet (Bartholomeu et al. 2005) and R2 Intelligence Test evaluates the abstract intellectual content, not including all the intellectual potential of students. The results will be presented in percentual values (%).

Biological samples

Study group: The blood samples (n= 20) were collected for the following analyses: metals, biomarkers of oxidative stress, hematological parameters, hepatic enzymes, urea, creatinine and butyrylcholinesterase activity (BuChe). The urine samples were collected at two moments. At first moment, urine samples (n= 20) were collected for determination of cotinine, an indicator of tobacco exposure. At second moment, one year after the first collection, a new collect was realized and the urine was used for the

measurement of renal function through of quantification of urinary protein (proteinuria) and for the qualitative examination of urine (EQU), due to a suspicion of nephrotoxicity in thirteen children after prior clinical evaluation. In addition, analysis of metals was performed in the hair of these children (n= 13).

Control group: The blood samples (n= 20) were collected for analysis of oxidative stress biomarkers in this group. For this, the blood was collected in EDTA tubes for quantification of MDA levels and in tubes with heparin for dosage of ALA-D activity and ALA-D reactivation index.

Urine

Urine samples (20 mL) were collected for cotinine determination and at second moment were collected (20 mL) for analysis of proteinuria and EQU. The samples were stored in polyethylene bottles and refrigerated at -4 ºC until further analysis.

Blood

Venous blood samples (10 mL) were collected into EDTA tubes (Trace Metal Free), heparin tubes and tubes without anticoagulant. Plasma and serum were obtained by centrifugation of 1500 g for 10 min. Plasma was used to analyze the MDA levels. Serum was used for quantification of creatinine, urea, hepatic enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and ɣ-glutamyl transferase (GGT) and BuChe activity. Whole blood with EDTA was used for determination of hemoglobin (Hb), hematocrit (Ht) and metals and the one with heparin for quantification of ALA-D activity and ALA-D reactivation.

Hair

Hair samples were collected with a stainless steel scissors of high quality (surgical), removing from the occipital region, just above the neck, a strand of hair weighing between 250 and 500 mg. Only the three centimeters were used for analysis. The samples were stored in polyethylene tubes, identified by a label on it with the children's name and date of collection.

Laboratory analyses

Hematological and biochemical analyses

Hemoglobin (Hb) and hematocrit (Ht) were determined through ABX Pentra 80 (Hematology Analyzer – Diamond Diagnostics, USA).

Biochemical analyses were determined by humid chemistry using the device Cobas Integra® 400 plus (Roche, Indianapolis, USA), utilizing commercial laboratory kits. Hepatic and renal functions were determined in serum by quantification of the enzymes AST, ALT and GGT and concentrations of creatinine and urea, respectively. In addition, the renal function was also determinated in urine by proteinuria and EQU.

Butyrylcholinesterase (BuChe) is an enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine, a key process in the regulation of the cholinergic system (Garcia et al. 2008). BuChE activity in serum was determined by commercial kits (Doles reagents, Goiânia, GO, Brazil) according to the method of Ellman et al. (1961).

Cotinine

Cotinine is a metabolite of nicotine and is a suggested with an ideal biomarker to evaluate tobacco exposure. The urinary cotinine was analyzed by high performance liquid chromatography (HPLC) according to the method of Cattaneo et al. (2006).

Determination of metals

Metals in blood

The toxic metals measured in whole blood (n= 20) of children in the study group were Lead (Pb), Arsenic (As), Cadmium (Cd), Nickel (Ni) and Manganese (Mn) and the essential metals determined in whole blood were Selenium (Se), Copper (Cu) and Cobalt (Co). The determination of metals levels were performed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), according to the method of Nunes et al. (2010). Briefly, into (15 mL) conical tubes, 200 µL of blood samples was mixed with 500 µL of 10% v/v tetramethylammonium hydroxide (TMAH) solution, incubated for 10 min, and subsequently diluted to 10 mL with a solution containing 0.05% w/v ethylenediamine tetraacetic acid (EDTA) + 0.005% v/v Triton X-100. After that, samples were directly analyzed by ICP-MS.

Metals in hair

The toxic metals that were measured in children (n= 13) in this biological sample were Aluminum (Al), Mercury (Hg), Lead (Pb), Cadmium (Cd), Arsenic (As), Silver (Ag) and Nickel (Ni) and the essential metals determined were Selenium (Se), Copper (Cu) and Cobalt (Co). This analysis was performed by ICP-MS and the equipment used was mod. ELAN 5000 (Perkin Elmer-Sciex).

The methodology used for hair washing, acid digestion and subsequent determination of elements of interest by ICP-MS was the method described by Fortes (1999). First, hair has washed successively with acetone/EXTRAN® (1% v/v)/Milli-Q water in an ultrasound bath to eliminate of exogenous elements. Then, it was dryed at 60ºC in an oven, a digestion of 200 to 250 mg of sample was carried with 2.5 mL of HNO_3 (sub-bidistilled) and 1.0 mL of H_2O_2 (supra pure) in a polypropylene tube with threaded cover in block digester at 70ºC, this was diluted to a final volume of 25 mL and metals were determined by ICP-MS.

Analysis of biomarkers of oxidative stress

Quantification of lipid peroxidation was performed through measurement of MDA levels by HPLC with VIS detection, as described by our group (Grotto et al. 2007). This method analyzes the MDA levels after alkaline hydrolysis.

Activity and reactivation index of δ-aminolevulinate dehydratase (ALA-D) were determined in whole blood according to the method of Sassa (1982) with some modifications. The enzyme activity was determined by rate of porphobilinogen (PGB) formation in 1 h at 37°C, in the presence and absence of the reducer agent dithiotreitol (DTT – 2 mM final concentration). The enzyme reaction was initiated after 10 min of pre-incubation. The reaction was started by adding δ-aminulevulinic acid (ALA) to a final concentration of 4 mM in phosphate buffered solution at pH 6.8, and incubation was carried out for 1 h at 37º C and the reaction product was measured at 555 nm. The reactivation index was estimated using: A-B/A*100 where A= absorbance of assay with DTT and B= absorbance of assay without DTT.

Water analysis

Analysis of metals in drinking water was held in the houses of eleven children in the study group – two children lived in the same house - the same children that performed hair analysis to verify whether water could be a source of contamination. The concentrations of metals in drinking water were expressed in ppm ($mg.L^{-1}$) and the equipment used was an ICP-MS model ELAN 5000 from Perkin Elmer/Sciex (USA).

Calibration was performed using standard solutions at a concentrations of 1.0 $mg.L^{-1}$ (Perkin Elmer 29 and Merck Titrisol) and acidified with bidistilled nitric acid. The concentrations of the calibration curve ranged from 10 $mg.L^{-1}$ to 100 $mg.L^{-1}$, and the internal standard of calibration was Rh at a concentration of 10 $mg.L^{-1}$ used for calibration. The limit of detection (LOD) was calculated using the formula LOD= 3x (SD/S) and limit of quantification (LOQ) was determined by the formula LOQ= 10x (SD/S), where SD represents the standard deviations of the reading of 10 whites and S is the sensitivity of the analytical curve (slope).

Statistical Analysis

Analysis of the data was performed using the software Statistica version 6.0 and the results were expressed as mean ± standard error (SE). Mann-Whitney test was used due to non-normal distribution of variables to verify statistical differences between groups and Spearman test was used to evaluate correlations between variables. Results with $p< 0.05$ were considered significant.

Results

For cognitive assessment the Bender Test and R2 Intelligence Test were applied in children of the study group. Among the 20 children evaluated, 18 (90%) were below average for the Bender Test and only 2 children were normal (10%) for the test. For R2 Intelligence Test, 3 (15%) children showed poor intelligence, 11 (55%) showed mean intelligence, 5 (25%) children showed limit intelligence and 1 (5%) children show above-average intelligence.

Biochemical and hematological parameters are shown in (**Table 1**). Hematological parameters such as Ht and Hb were within the reference values. Biochemical parameters such as urea, creatinine, AST, ALT and GGT also were within the reference values. In addition, results of serum BuChe activity were also showed normal and the mean urinary concentrations of cotinine in children showed <10 $ng.mL^{-1}$, being that the mean cotinine concentrations in urine for non-smokers and passive smokers is between 8 – 25 $ng.mL^{-1}$ (DFG 2001).

The concentrations of toxic elements found in blood are shown in (**Table 2**). Among the metals assessed in this biological sample, only the blood lead was found to be above the acceptable concentration (<25 $\mu g.dL^{-1}$). In addition, the children presented deficient levels of selenium according to (**Table 3**), which shows the levels of essential metals found in the blood of children.

Besides, the mean concentration levels of toxic elements in the hair samples of children with suspicion of nephrotoxicity are shown in (**Table 4**). In this biological sample, results show a high concentration of aluminum. For evaluation of renal function, these children showed proteinuria within the reference values (data not shown) except for one child whose proteinuria was 33.3 $mg.dL^{-1}$, above the reference values (<

11.9 mg.dL^{-1}) and in EQU the presence of proteins and some epithelial cells was found in the sediment. The mean concentration levels of essential elements in these samples are show in (**Table 5**). The mean concentration of selenium found in hair of children was below the reference values confirming the deficiency of this element also found in the blood. The drinking water analysis (**Table 6**) in homes of children showed high concentrations of aluminum.

Moreover, the biomarker of lipid peroxidation was significantly increased in study group when compared with control group, with plasma MDA levels being 6.50 ± 0.18 µmol.L^{-1} vs. 3.85 ± 0.19 µmol.L^{-1} ($p< 0.05$), respectively. ALA-D activity showed no significant difference between both groups ($p> 0.05$) being 20.83 ± 1.67 U.L^{-1} (study group) vs. 21.33 ± 1.19 U.L^{-1} (control group) and the ALA-D reactivation index was significantly higher in study group when compared to the children of control group, being 57.12 ± 10.25% vs. 26.65 ± 3.72% ($p< 0.05$), respectively, according to (**Figure I**).

Spearman correlations were performed and a significant negative correlation was observed ($p <0.05$) between concentrations of blood lead versus the blood ALA-D activity (**Figure II**) and a significant positive correlation between concentrations of blood lead and the ALA-D reactivation in study group (**Figure III**). In addition, (**Figure IV**) shows that MDA levels were negatively correlated with ALA-D activity in study group. Moreover, there was no correlation between aluminum levels in hair of children assessed for this biological sample versus biomarkers of oxidative stress and versus blood lead levels in these children.

Discussion

Children have special vulnerabilities to the toxic effects of chemicals and children's exposure to chemicals at critical stages in their physical and cognitive development may have severe long-term consequences for health. The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) have identified a growing number of children's health impacts from exposure to chemicals such as behavioral disorders, learning disabilities, autism and neurological impairments (Lloyd-Smith and Sheffield-Brotherton 2008).

Most children had below average results in psychomotor evaluation, in terms of visomotor maturation. The poor performance on the Bender Test corroborates with the bad performance on R2 Intelligence Test, since it is known that the use of Bender Test has been used for detecting the maturity to learning, considering that a low level of perceptual-motor maturation may contribute for the appearance of learning problems (Bartholomeu et al. 2005). In other words, the precarious development of cognitive functions are the result of visomotor immaturity. Moreover, several factors may be involved in the learning difficulties of these children, such as a lack of stimulation from the poor environment where that they live, lack of family encouragement and also low nutrition food, as shown by deficiency of selenium in blood and hair. It is know that selenium is an essential metal for normal functioning of nervous functions and selenium deficiency has been associated with both cognitive and neuromotor impairment (Lemire et al. 2011).

In addition, the determination of the serum activity of BuChe and urinary cotinine concentration were performed to assess the exposure to pesticides and nicotine, respectively. These analyses were relevant because the region of Agudo, RS, Brazil,

where studied children live, is characterized by the cultivation of tobacco and the common use of pesticides (Bortoluzzi et al. 2006). In addition, it is know that tobacco contains lead and other heavy metals (Willers et al. 2005). However, at the time of collection, no children had low activity of BuChe and high urinary cotinine concentrations, indicating no exposure to these chemicals in this period.

Among the metals studied, the mean concentration of blood lead found in this study was higher than recommended for some international institutions such as WHO and Centers for Disease Control and Prevention (CDC) for children in the USA. These institutions recommend that public health actions be initiated when the blood lead levels are above 10 $\mu g.dL^{-1}$ in preschool children. Furthermore, lead has a historical particular importance among environmental neurotoxicants (Landrigan et al. 1998).

Lead is one of the most studied toxic metals and this metal remains indefinitely in contaminated soil, being the exposure to it a public health problem, because even low lead levels can result in learning difficulties, low levels of growth and induce IQ deficits and attention dysfunction (Selevan et al. 2003; Costa de Almeida et al. 2010). Currently, no limit for lead that induces neurobehavioral effects has been established, but cognitive deficits were reported in children with blood lead levels below 5 $\mu g.dL^{-1}$ (Torrente et al. 2005) and in this research was found levels four times higher were found, which is an alarming data.

The relationship between lead exposure and learning disorders and other intellectual deficits is supported by numerous studies. Kim et al. (2010) shows that blood lead concentrations are associated with difficulties in sustained attention and learning, confirming others studies indicating that children exposed to low levels of lead may have learning difficulties (Canfield et al. 2004; Lyngbye et al. 1990).

Moreover, hair has been used to determine metals concentrations since it can be easily collected, transported and stored. Also reflects longer periods of exposure comparing to blood (Zaida et al. 2007). In our results, analyses in hair for toxic metals indicated that aluminum was above the reference values for this sample in eleven children. Aluminum is extensively used in modern daily life and its exposure is inevitable due to its abundance on Earth's crust, being present in foods and beverages, medications, used in cookware, etc (Nayak et al. 2002; Nayak et al. 2010). According to *Doctor's Data, Inc*, high levels of aluminum in the hair are found in children with behavioral/learning disorders, such as attention deficit/hyperactivity disorder (ADHD) and autism. Although there are no reports of acute toxicity of Al in people exposed to normal levels of metal, several studies have evaluated whether chronic exposure can have effects on long-term health (Campbell et al. 2004).

Furthermore, aluminum levels were also high in drinking water analyzed in homes of children who had high concentrations of this metal in hair, considering water as a possible source of contamination to metals. The Brazilian legislation (National Health Foundation - FUNASA - Ministry of Health) establishes procedures and responsibilities relating to control and surveillance of water quality for human consumption and pattern of drinking. For aluminum, the maximum allowed for human consumption is 0.2 $mg.L^{-1}$.

In addition, oxidative stress is the possible mechanism involved in the toxicity induced by some metals, such as lead and aluminum (Flora et al. 2009; Kumar and Gill 2009; Nayak et al. 2010). This process is a consequence of an imbalance between oxidant species and antioxidants in the body. The oxidative damage caused by lead toxicity can be caused by two independent mechanisms (Ercal et al. 2001). The first involves the direct formation of reactive oxygen species (ROS) including singlet

oxygen, hydrogen peroxides and hydroperoxides and the second mechanism is achieved via depletion of the cellular antioxidant pool (Gurer and Ercal 2000). Aluminum has been reported to enhance peroxidative damage to lipids, proteins and decreases antioxidant enzyme status (Jyoti et al. 2007).

The parameters of oxidative stress were elevated in children with learning disorders when compared with control group. These results are evidenced by increased levels of MDA, a biomarker of lipid peroxidation and the increase in ALA-D reactivation index, demonstrating that oxidative stress can modify the activity of this enzyme. The increase in the ALA-D reactivation index may be related to an overproduction of free radicals, confirmed by an increase in MDA production (Valentini et al. 2008). Al-Gadani et al. (2009) demonstrated increased levels of MDA in autistic children, reflecting a relationship between lipid peroxidation and cognitive development. Also, Zoroglu et al. (2004) reported an increase in TBARS levels in the serum of autism patients.

δ-Aminolevulinic acid dehydratase (ALA-D) is the second enzyme in the heme biosynthesis pathway and catalyzes condensation of two molecules of 5-aminolevulinate to produce porphobilinogen, which is the precursor of porphiryns. ALA-D is a zinc metalloenzyme possessing thiol (SH) groups, which are essential for its activity (Jin et al. 2006). The inhibition of the enzyme may affect the heme biosynthesis pathway and may have pathological consequences. It is known that lead has an affinity for SH groups and inhibits ALA-D activity. Our results demonstrated a strong positive relation between lead levels and index of reactivation of the ALA-D indicating the inhibition of the ALA-D activity due to linking of lead to SH-groups. In addition, ALA-D inhibition results in accumulation of aminolevulinic acid (ALA). Therefore, ALA-D is sensitive to the effects of lead and it implies that ALA-D activity

might be a promising indicator of lead-induced oxidative damage in red blood cells (RBCs) (Gurer-Orhan et al. 2004).

Moreover, the present study showed correlations between the increased of lead concentrations in blood and decreased of ALA-D activity. ALA-D is an indicator of lead intoxication because the inhibition contributes to the development of oxidative stress due to ALA accumulation. ALA may rapidly oxidize to generate ROS such as superoxide ion, hydroxyl radical and hydroxyl peroxides (Reckziegel et al. 2011). Furthermore, the increased circulating of ALA, a weak gamma-aminobutyric acid (GABA) agonist, decreases GABA release by presynaptic inhibition and may account for some of the behavioral disorders seen in lead toxicity (Needlemann 2004). In addition, correlation between increased MDA levels in study group and decreased of ALA-D activity showed that an imbalance between oxidants and antioxidants is present.

However, there were no correlations between high levels of aluminum in hair of study group and the parameters of oxidative stress. Also, no correlation was found between increased levels of aluminum in the hair and increasing concentrations of lead in blood, showing that exposure to these metals do not have the same source.

Conclusion

The results of this study showed the presence of high concentrations of toxic metals such as lead and aluminum in blood and hair, respectively, of children who had learning disabilities, confirmed by cognitive evaluation. According to the results of this study, the source of aluminium contamination can be the drinking water. In addition, our results showed a selenium deficiency in these children, which contributes to the cognitive impairment. Furthermore, we suggest that the process of oxidative stress is involved in the mechanism of toxicity caused by exposure to these metals. However, further studies in this region are necessary to confirm the sources of contamination for these metals, mainly lead. Furthermore, more studies are needed to determine other causes to explain the learning difficulties of these children, such as poor nutrition or lack of family encouragement, so they can take preventive measures to avoid the problem of cognitive impairment and other long-term damage to the health of these children.

References

Ahamed M, Siddiqui MK. 2007. Low level lead exposure and oxidative stress: current opinions. Clinica chimica acta; international journal of clinical chemistry. 383:57-64.

Al-Gadani Y, El-Ansary A, Attas O, Al-Ayadhi L. 2009. Metabolic biomarkers related to oxidative stress and antioxidant status in Saudi autistic children. Clinical biochemistry. 42:1032-1040.

Bartholomeu D, Rueda FJ and Sisto FF. 2005. Teste de Bender e dificuldades de aprendizagem: Quão válido é o sistema Koppitz?. Avaliação Psicologica. 4(1):13-21.

Bortoluzzi EC, Rheinheimer DS, Gonçalves CS, Pellegrini JBR, Zanella R and Copetti ACC. 2006. Contaminação de águas superficiais por agrotóxicos em função do uso do solo numa microbacia hidrográfica de Agudo, RS. Revista Brasileira de Engenharia Agrícola e Ambiental. 10(4):881-887.

Campbell A, Becaria A, Lahiri DK, Sharman K, Bondy SC. 2004. Chronic exposure to aluminum in drinking water increases inflammatory parameters selectively in the brain. Journal of neuroscience research. 75:565-572.

Canfield RL, Gendle MH, Cory-Slechta DA. 2004. Impaired neuropsychological functioning in lead-exposed children. Developmental neuropsychology. 26:513-540.

Cattaneo R, Alegrett AP, Sagebin FR, Abreu CM, Peterson GO, Thiesen FV. 2006. Validação do método para determinação de cotinina em urina por cromatografia líquida de alta eficiência. Revista Brasileira de Toxicologia. 19:25-31.

Chen IC, Lin CH, Wen SH, Wu CH. 2007. How Effectively Do Parents Discern Their Children's Cognitive Deficits at a Preschool Age?. Journal of the Chinese Medical Association. 74(6):445-450.

Costa de Almeida GR, de Freitas Tavares CF, de Souza AM, Sampaio de Sousa T, Rodrigues Funayama CA, Barbosa F, Jr., Tanus-Santos JE, Gerlach RF. 2010. Whole blood, serum, and saliva lead concentrations in 6- to 8-year-old children. The Science of the total environment. 408:1551-1556.

Deutsche Forschungsgmeinschaft (DFG). List of MAK and BAT values. 2001. Weinhaim.

Doctor's Data Inc. [cited 2011 May 18]. Available from: http://www.doctorsdata.com/home.asp.

Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 7:88–95.

Ercal N, Gurer-Orhan H, Aykin-Burns N. 2001. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Current topics in medicinal chemistry. 1:529-539.

Flora, SJ. 2009. Structural, chemical and biological aspects of antioxidants for strategies againstmetal andmetalloid exposure. Oxidative Medicine and cellular longevity. 2:191–206.

Fortes LMC. 1999. Tese de Doutorado. Pontificia Universidade Catolica do Rio de Janeiro. Rio de Janeiro, RJ.

Garcia SC, Wyse ATS, Valentini J, Roehrs M, Moro AM, Paniz C, Schmitt G, Grotto D, Pomblum VJ. 2008. Butyrylcholinesterase activity is reduced in haemodialysis patients: Is there association with hyperhomocysteinemia and/or oxidative stress?. Clinical Biochemistry. 41:474–479.

Grotto D, Santa Maria LD, Boeira S, Valentini J, Charão MF, Moro AM, Nascimento PC, Pomblum VJ, Garcia SC. 2007. Rapid quantification of malondialdehyde in plasma

by high performance liquid chromatography-visible detection. Journal of pharmaceutical and biomedical analysis. 43(2):619-624.

Gurer H, Ercal N. 2000. Can antioxidants be beneficial in the treatment of lead poisoning? Free radical biology & medicine. 29:927-945.

Gurer-Orhan H, Sabir HU, Ozgunes H. 2004. Correlation between clinical indicators of lead poisoning and oxidative stress parameters in controls and lead-exposed workers. Toxicology. 195:147-154.

Heitland P, Koster HD. 2006. Biomonitoring of 37 trace elements in blood samples from inhabitants of northern Germany by ICP-MS. Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements. 20:253-262.

Jin Y, Liao Y, Lu C, Li G, Yu F, Zhi X, Xu J, Liu S, Liu M, Yang J. 2006. Health effects in children aged 3-6 years induced by environmental lead exposure. Ecotoxicology and environmental safety. 63:313-317.

Jyoti A, Sethi P, Sharma D. 2007. Bacopa monniera prevents from aluminium neurotoxicity in the cerebral cortex of rat brain. Journal of ethnopharmacology. 111:56-62.

Kim Y, Cho SC, Kima BN, Hong YC, Shin MS, Yoo HJ, Kima JW, Bhang SY. 2010. Association between blood lead levels (<5 μg/dL) and inattention-hyperactivity and neurocognitive profiles in school-aged Korean children. Science of the Total Environment. 408:5737–5743.

Kordas K, Queirolo EI, Ettinger AS, Wright RO, Stoltzfus RJ. 2010. Prevalence and predictors of exposure to multiple metals in preschool children from Montevideo, Uruguay. The Science of the total environment. 408:4488-4494.

Kumar V, Gill KD. 2009. Aluminium neurotoxicity: neurobehavioural and oxidative aspects. Archives of toxicology. 83:965-978.

Landrigan PJ, Carlson JE, Bearer CF, Cranmer JS, Bullard RD, Etzel RA, Groopman J, McLachlan JA, Perera FP, Reigart JR, Robison L, Schell L and Suk WA. 1998. Children's health and the environment: a new agenda for prevention research. Environmental health perspectives. 106(3):787-794.

Lemire M, Fillion M, Frenette B, Passos CJ, Guimaraes JR, Barbosa F, Jr., Mergler D. 2011. Selenium from dietary sources and motor functions in the Brazilian Amazon. Neurotoxicology.

Lloyd-Smith M, Sheffield-Brotherton B. 2008. Children's environmental health: intergenerational equity in action--a civil society perspective. Annals of the New York Academy of Sciences. 1140:190-200.

Lyngbye T, Hansen ON, Trillingsgaard A, Beese I, Grandjean P. 1990. Learning disabilities in children: significance of low-level lead-exposure and confounding factors. Acta Paediatrica Scandinavica. 79(3):352–60.

Moodie SM, Tsui EK, Silbergeld EK. 2010. Community- and family-level factors influence care-giver choice to screen blood lead levels of children in a mining community. Environmental research. 110:484-496.

Nayak P, Sharma SB, Chowdary NV. 2010. Augmentation of aluminum-induced oxidative stress in rat cerebrum by presence of pro-oxidant (graded doses of ethanol) exposure. Neurochemical research. 35:1681-1690.

Nayak P. 2002. Aluminum: impacts and disease. Environmental research. 89: 101-115.

Needleman H. 2004. Lead poisoning. Annual review of medicine. 55:209-222.

Nunes JA, Batista BL, Rodrigues JL, Caldas NM, Neto JA, Barbosa F, Jr. 2010. A simple method based on ICP-MS for estimation of background levels of arsenic, cadmium, copper, manganese, nickel, lead, and selenium in blood of the Brazilian population. Journal of toxicology and environmental health. 73 (A):878-887.

Oga S, Camargo MA, Batistuzzo JO. 2008. Fundamentos de Toxicologia. 3 ed. Atheneu Editora.

Pastura GM, Mattos P, Araújo AP. 2005. Desempenho escolar e transtorno de déficit de atenção e hiperatividade. Revista de Psquiatria Clínica. 32:324-329.

Reckziegel P, Dias VT, Benvegnu D, Boufleur N, Silva Barcelos RC, Segat HJ, Pase CS, Dos Santos CM, Flores EM, Burger ME. 2011. Locomotor damage and brain oxidative stress induced by lead exposure are attenuated by gallic acid treatment. Toxicology letters. 203:74-81.

Rodrigues JL, Batista BL, Nunes JA, Passos CJ, Barbosa F, Jr. 2008. Evaluation of the use of human hair for biomonitoring the deficiency of essential and exposure to toxic elements. The Science of the total environment. 405:370-376.

Sassa S. δ-Aminolevulinic acid dehydratase assay. 1982. Enzyme. 28:133–45.

Selevan SG, Rice DC, Hogan KA, Euling SY, Pfahles-Hutchens A, Bethel J. 2003. Blood lead concentration and delayed puberty in girls. The New England journal of medicine. 348:1527-1536.

Silva CA, Fruchtengarten L. 2005 Riscos químicos ambientais à saúde da criança. Jornal de Pediatria. 81:205-221.

Siqueira CM, Gurgel-Giannetti J. 2011. Poor school performance: an updated review. Revista da Associacao Medica Brasileira. 57:78-87.

Surkan PJ, Zhang A, Trachtenberg F, Daniel DB, McKinlay S, Bellinger DC. 2007. Neuropsychological function in children with blood lead levels <10 microg/dL. Neurotoxicology. 28:1170-1177.

Torrente M, Colomina MT, Domingo JL. 2005. Metal concentrations in hair and cognitive assessment in an adolescent population. Biological trace element research. 104:215-221.

Valentini J, Grotto D, Paniz C, Roehrs M, Burg G, Garcia SC. 2008. The influence of the hemodialysis treatment time under oxidative stress biomarkers in chronic renal failure patients. Biomedicine & Pharmacotherapy. 62:378-382.

Willers S, Gerhardsson L, Lundh T. 2005. Environmental tobacco smoke (ETS) exposure in children with asthma-relation between lead and cadmium, and cotinine concentrations in urine. Respiratory medicine. 99:1521-1527.

Yumoto S, Nagai H, Matsuzaki H, Matsumura H, Tada W, Nagatsuma E, Kobayashi K. 2001. Aluminium incorporation into the brain of rat fetuses and sucklings. Brain research bulletin. 55:229-234.

Zaida F, Chadrame S, Sedki A, Lekouch N, Bureau F, Arhan P, Bougle D. 2007. Lead and aluminium levels in infants' hair, diet, and the local environment in the Moroccan city of Marrakech. The Science of the total environment. 377:152-158.

Zoroglu SS, Armutcu F, Ozen S, Gurel A, Sivasli E, Yetkin O, Meram I. 2004. Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism. European archives of psychiatry and clinical neuroscience. 254:143-147.

Tables

Table 1. Biochemical and hematological parameters of the children with learning disabilities.

Parameters	*Study group (n= 20)*	*Reference values**
Ht (%)	40.18 ± 0.69	35 - 45 %
Hb (g.dL^{-1})	13.44 ± 0.25	12 - 15 g.dL^{-1}
AST (U.L^{-1})	24.00 ± 1.09	10 – 40 U.L^{-1}
ALT (U.L^{-1})	15.15 ± 1.02	10 - 35 U.L^{-1}
GGT (U.L^{-1})	13.95 ± 4.63	≤ 50 U.L^{-1}
Urea (mg.dL^{-1})	24.42 ± 1.80	13 – 47 mg.dL^{-1}
Creatinine (mg.dL^{-1})	0.37 ± 0.02	0.3 – 0.7 mg.dL^{-1}
BuChe (U.I. mL^{-1})	9.90 ± 0.40	5.0 – 12.1 U.I.mL^{-1}

Results expressed as mean ± standard error (SE).

*Burtis, C.A.; Ashwood, E.R.; Bruns, D.E. The Fundamentals of Clinical Chemistry, 6th ed, 2008.

Table 2. Toxic metals concentrations in blood of children with learning disabilities.

Metals	*Study group (n= 20)*	*Reference value**
Lead (Pb)	42.16 ± 8.70 μg.dL^{-1}	< 25 μg.dL^{-1}
Arsenic (As)	3.93 ± 0.08 μg.L^{-1}	0.2 - 6.2 μg.L^{-1}
Cadmium (Cd)	0.11 ± 0.07 μg.L^{-1}	0.10 – 1.10 μg.L^{-1}
Manganese (Mn)	11.11 ± 0.55 μg.L^{-1}	1.5 – 22.0 μg.L^{-1}
Nickel (Ni)	4.62 ± 1.40 μg.L^{-1}	1.0 – 28.0 μg.L^{-1}

Results expressed as mean ± standard error (SE).

*Burtis, C.A.; Ashwood, E.R.; Bruns, D.E. The Fundamentals of Clinical Chemistry, 6th ed, 2008.

Table 3. Essential metals concentrations in blood of children with learning disabilities.

Metals	*Study group (n= 20)*	*Reference value**
Selenium (Se)	7.35 ± 0.22 μg.dL^{-1}	58 - 234 μg.dL^{-1}
Copper (Cu)	105.33 ± 2.64 μg.dL^{-1}	80 - 160 μg.dL^{-1}
Cobalt (Co)	0.17 ± 0.01 μg.L^{-1}	0.11 – 0.45 μg.L^{-1}

Results expressed as mean ± standard error (SE).

*Burtis, C.A.; Ashwood, E.R.; Bruns, D.E. The Fundamentals of Clinical Chemistry, 6th ed, 2008.

Table 4. Toxic metals concentrations in hair of children with learning disabilities.

Metals	*Study group (n= 13)* (μg.g^{-1})	*Reference value* (μg.g^{-1})*
Aluminum (Al)	47.61 ± 8.20	< 14
Mercury (Hg)	0.2 ± 0.03	< 2.3
Lead (Pb)	1.97 ± 0.57	< 9.3
Cadmium (Cd)	0.05 ± 0.008	< 0.3
Arsenic (As)	0.08 ± 0.008	< 0.15
Silver (Ag)	0.38 ± 0.22	< 0.4

Results were expressed as mean ± standard error (SE).

*Laboratory of ICP-MS and ICP-AES, Chemistry Department, PUC-RIO.

Table 5. Essential metals concentrations in hair of children with learning disabilities.

Metals	*Study group (n= 13)* ***(µg.g^{-1})***	*Reference value* ***(µg.g^{-1})****
Nickel (Ni)	0.31 ± 0.09	< 0.6
Selenium (Se)	0.63 ± 0.04	0.8 – 1.5
Copper (Cu)	12.88 ± 2.50	1.0 – 32.0
Cobalt (Co)	0.06 ± 0.01	0.003 – 0.03

Results were expressed as mean ± standard error (SE).

*Laboratory of ICP-MS and ICP-AES, Chemistry Department, PUC-RIO.

Table 6. Concentrations of metals in drinking water in house of study group (n= 10).

Metals	*Metals concentrations in houses of children* ***(ppm)***	*Reference values* ***(ppm)****
Aluminum (Al)	0.36 ± 0.08	0.2
Lead (Pb)	0.002 ± 0.0004	0.01
Mercury (Hg)	0.002 ± 0.0005	0.001
Cadmium (Cd)	0.001	0.005
Nickel (Ni)	0.002 ± 0.0023	-
Silver (Ag)	0.001	-
Arsenic (As)	0.001	0.01

Results were expressed as mean ± standard deviation (SD).

*According to Brazilian legislation of FUNASA (nº 1.469).

FIGURE CAPTIONS

Fig. I. Blood δ-aminolevulinate dehydratase activity (ALA-D) and index of δ-aminolevulinate dehydratase reactivation (ALA-RE) in study group (n= 20) and control group (n= 20).

*ALA-RE significantly different from control group ($p < 0.05$).

Fig. II. Spearman correlation between δ-aminolevulinate dehydratase enzyme (ALA-D) vs. blood lead levels (Pb) in the study group (n= 20).

Fig. III. Spearman correlation between index of δ-aminolevulinate dehydratase reactivation (ALA-RE) vs. blood lead levels (Pb) in the study group (n= 20).

Fig. IV. Spearman correlation between δ-aminolevulinate dehydratase enzyme (ALA-D) vs. lipid peroxidation biomarker (MDA) in the study group (n= 20).

FIGURES

Figure I

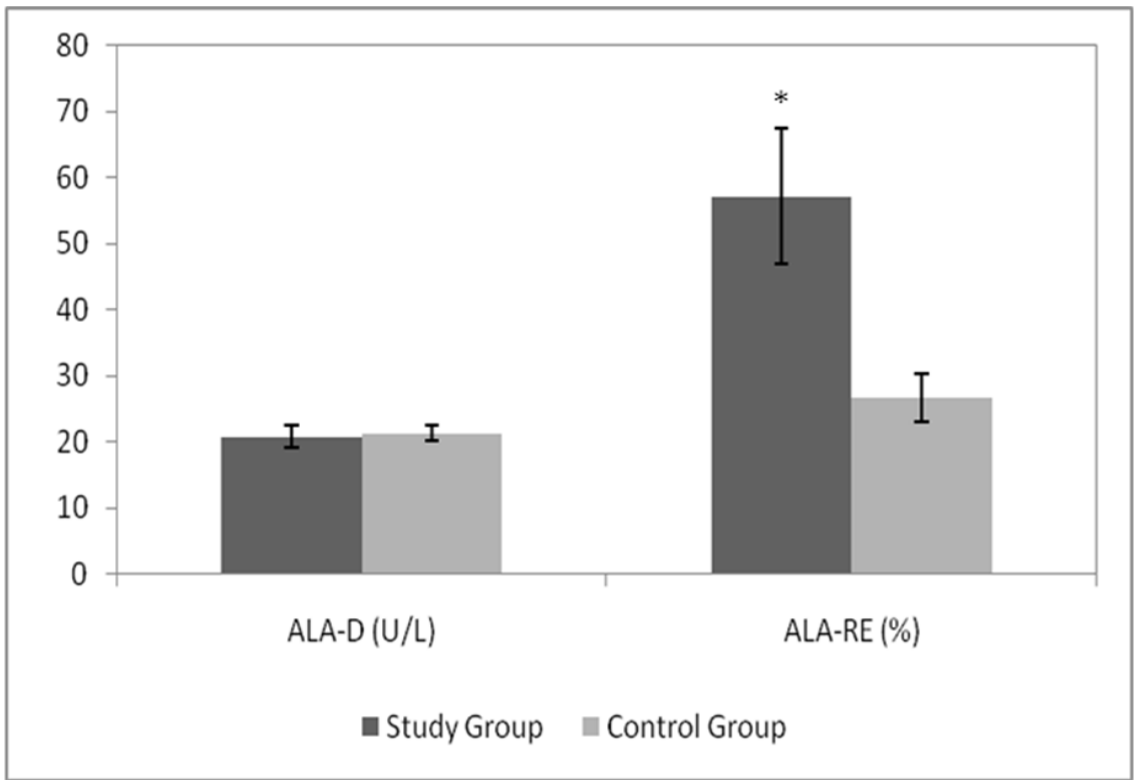


Figure II

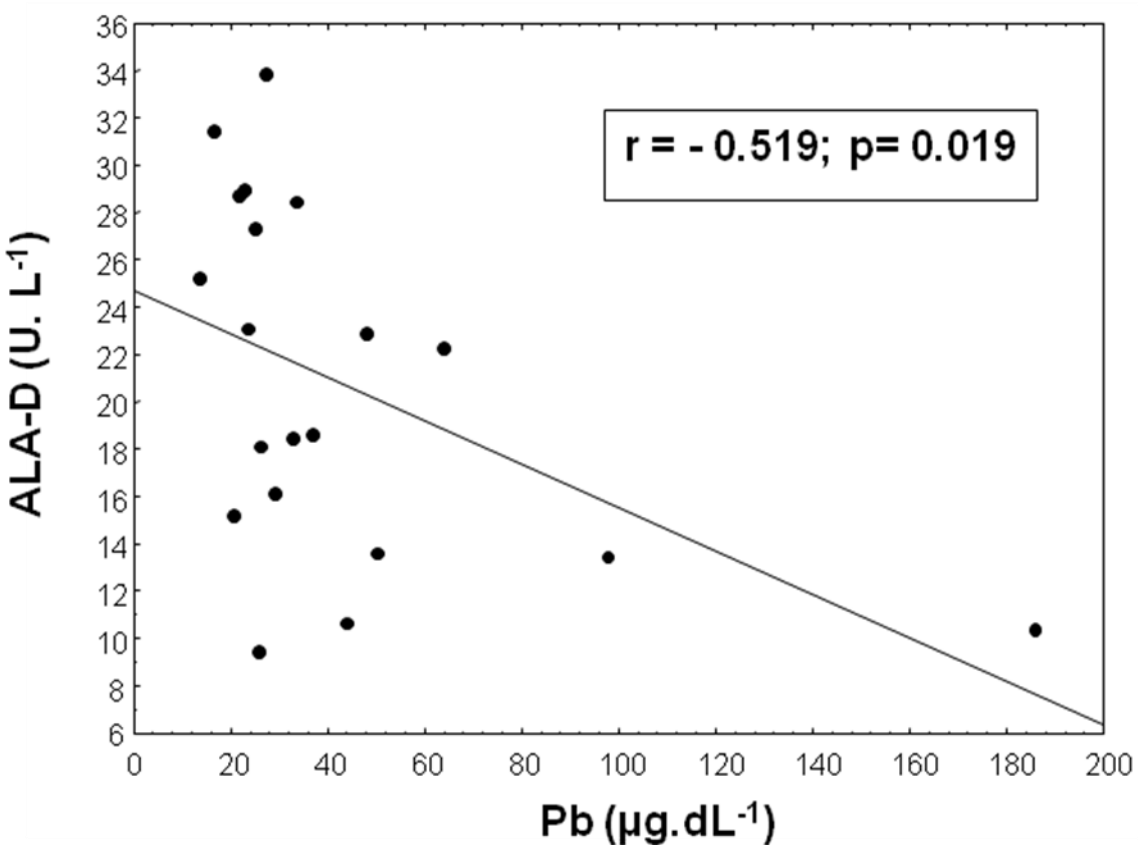

r = - 0.519; p= 0.019
ALA-D (U. L-1)
Pb (µg.dL-1)
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
0
20
40
60
80
100
120
140
160
180
200

Figure III

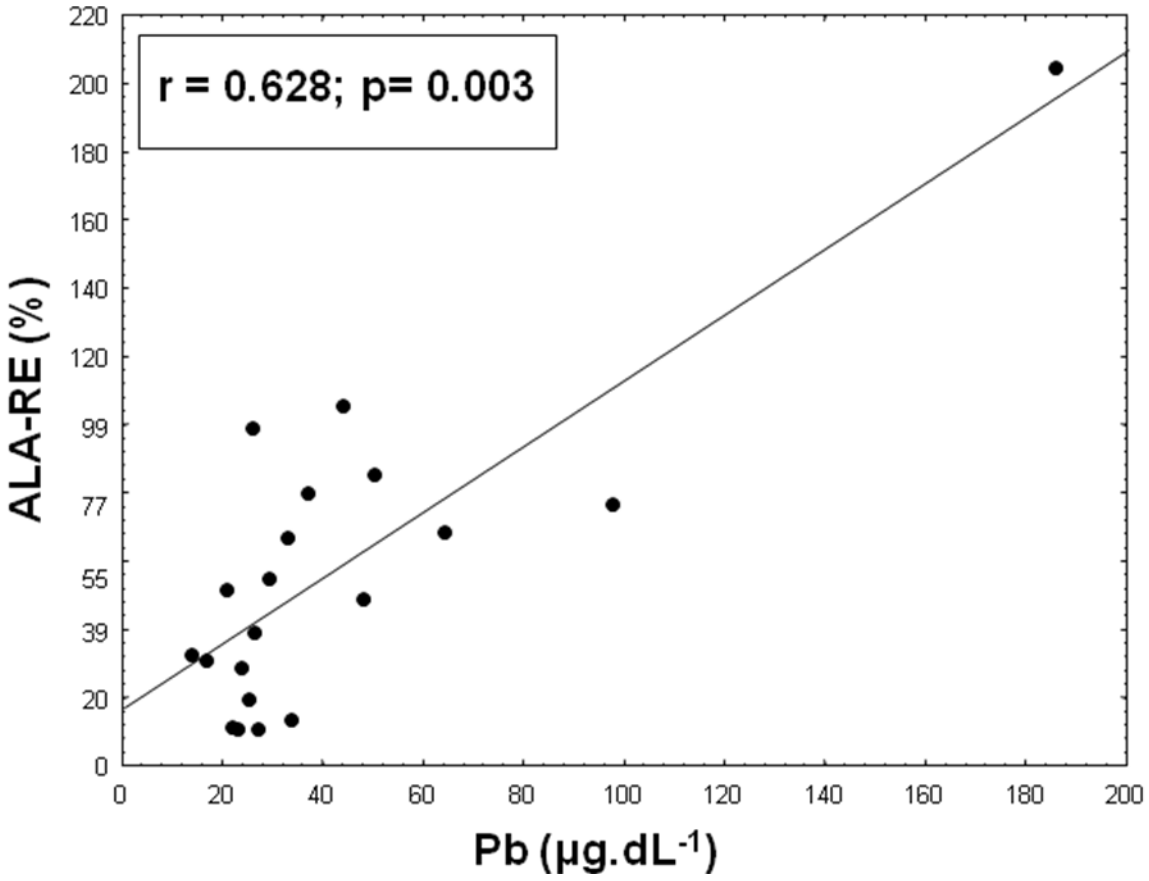
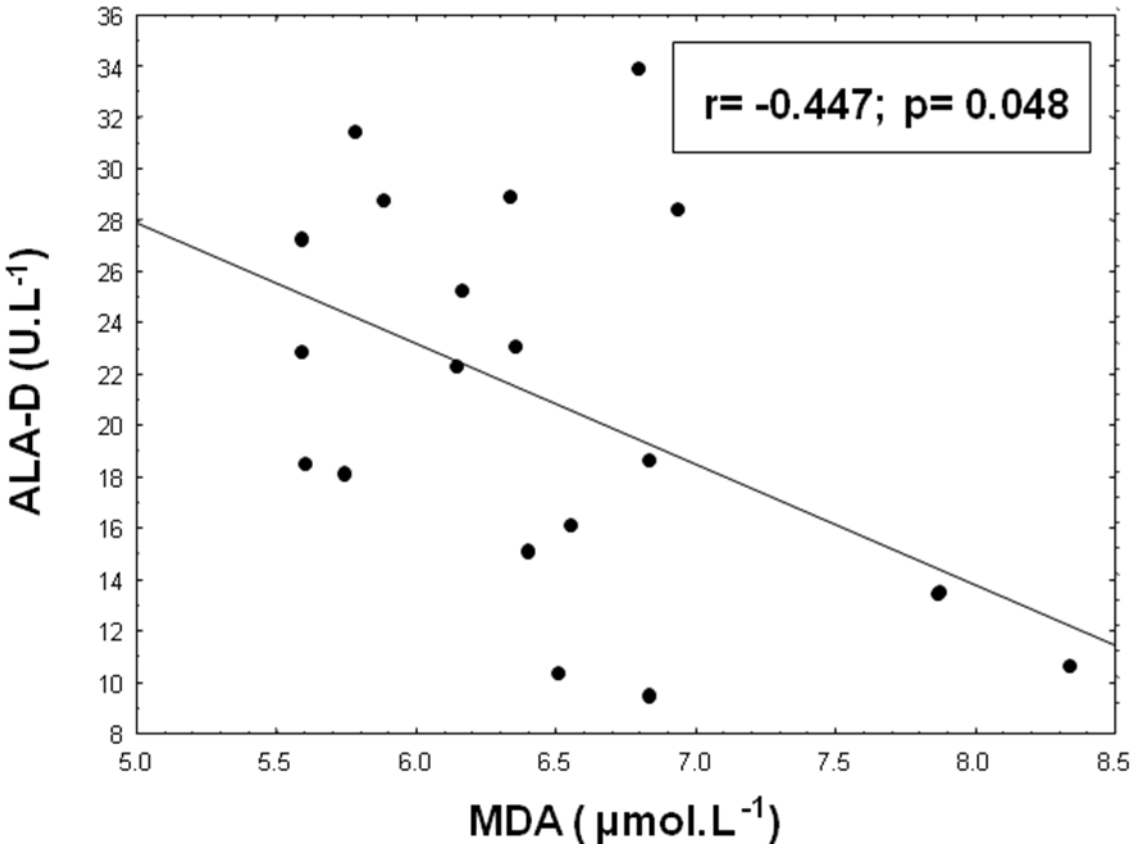

r = 0.628; p= 0.003
ALA-RE (%)
Pb (µg.dL-1)
220
200
180
160
140
120
99
77
55
39
20
0
0
20
40
60
80
100
120
140
160
180
200

Figure IV


r= -0.447; p= 0.048
ALA-D (U.L-1)
MDA (µmol.L-1)
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5

ANEXO

Normas para a publicação no "International Journal of Environmental Health Reserach".

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