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Environmental pathways and human exposure to manganese in southern Brazil

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ABSTRACT

The study of environmental pathways and human exposure to Manganese (Mn) in Southern Brazil was performed using two steps. The first step consisted of taking water samples from the surface of the Pardinho River. The average results from this technique showed a significant increase of pollutants, including increased levels of Mn, above the environmentally acceptable standard recommended by the Brazilian National Environment Council. Additionally, 64 soil samples were taken from areas with and without agricultural activity. Many results were above the mean crust and did not indicate significant differences of Mn levels between the sampled areas. For the second step, 12 families were selected and assessed for exposure to Mn in a region with high levels of Mn in the soil. Most of the analyzed foods contained amounts of Mn above the reference values, indicating that food can be an important source of exposure. The Mn content from the hair of most subjects studied was also high compared to reference values from non-exposed populations. Although the contamination appeared to come from a natural origin, the results found in the present study showed that the Mn levels present in the Pardinho River Basin are a relevant public health issue.

Key words: manganese, human exposure, environmental levels, Mn.

INTRODUCTION

Manganese (Mn) is one of the most abundant elements in the earth's crust, and average Mn concentrations in soils range between 500 and 900 mg.kg^{-1} (World Health Organization - WHO 1981). Mn is an essential element found in all living organisms and can also be found in the air and in most water supplies, though the principal mode of acquiring Mn is through food intake. In addition, Mn is a cofactor for a number of enzymatic reactions involved in phosphorylation, cholesterol and fatty acid synthesis (ATSDR 2000). Mn also plays an essential role in the immune system, in the regulation of cellular energy, in bone and connective tissue growth and in blood clotting. In the brain, Mn is an important cofactor for a variety of enzymes, including the

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antioxidant enzyme superoxide dismutase, and for enzymes involved in neurotransmitter synthesis and metabolism (Aschner et al. 2007).

Despite its vital nature, Mn has been known as a neurotoxin for at least 150 years (ATSDR 2000). Mn neurotoxicity was first identified as the cause of extrapyramidal syndrome in miners that were exposed to high concentrations of Mn while working in Mn mines. Chronic exposure to this metal can cause a neurodegenerative disease called "manganism", with symptoms that resemble Parkinson's disease (Barbeau 1984, Boudissa et al. 2006). The signs and symptoms of relatively high levels of exposure include postural instability, mood disorders and other psychiatric changes (i.e., depression, agitation, hallucinations) as well as parkinsonian symptoms, such as bradykinesia, rigidity, tremors, gait disturbance, postural instability and dystonia and/or ataxia. Cognitive deficits, such as memory impairment, reduced learning capacity, decreased mental flexibility, cognitive slowing and difficulty with processing visuo-motor and visuo-spatial information, have also been reported (Aschner et al. 2007).

It is not clear whether eating or drinking foods and liquids with excessive levels of manganese can cause symptoms of manganism (ATSDR 2000). Several reports have studied adverse effects after oral manganese exposure. These studies, which included children in their sample populations, have numerous limitations that preclude firm conclusions about the potential adverse effects from exposure to excess manganese. However, collectively, these studies have suggested that inhalation, ingestion of water and/or foodstuffs containing increased concentrations of manganese may result in adverse neurological effects (Kawamura et al. 1941, Kilburn 1987, Kondakis et al. 1989, Goldsmith et al. 1990, He et al. 1994, Iwami et al. 1994, Zangh et al. 1995, Alves et al. 1997, Batterman et al. 2011).

In the Pardinho River Basin area (RS, Brazil), previous studies have found excess Mn in soil (values ranging from 160 to 8,700 $mg.kg^{-1}$), food (values ranging 1.1 to 59.7 $mg.kg^{-1}$) and human blood samples (5.3% presented values over 50 $\mu g.L^{-1}$), indicating the possibility of environmental contamination that could be natural or due to agricultural practices (tobacco farming is the main agricultural activity in the area). In that region, there is a high manifestation of anxiety and depression cases associated with high serum levels of Mn (up to 800 $ng.mL^{-1}$), which confirmed the necessity of investigating the causes of high Mn levels in the rural area, including pesticide use. We highlight levels indicating 25 $ng.mL^{-1}$ or more are enough to be detected alterations in central nervous system due to contamination with manganes. (Hermes and Torres 2003).

This contamination hypothesis is due to the local agricultural practices associated with tobacco farming where high doses of pesticides are applied during the tobacco seedling cultivation. In the tobacco production, mancozeb, a dithiocarbamate pesticide containing manganese, has been used to treat blue mold and antracnose for many years. A typical usage of mancozeb is 135 g for 45 m^2, and it is applied 5 to 6 times per crop. Each molecule of this pesticide contains one manganese atom, which becomes incorporated into the soil due to the low mobility in that media.

Another standard, although not recommended, activity was that farmers would cultivate vegetables in areas that had previously been used to cultivate tobacco seedlings (Etges and Ferreira 2006). Accordingly, the authors of this paper asserted the necessity of investigating this situation. In that context, the present study is focused on assessing the environmental pathways and human exposure to Mn in the Pardinho River Basin.

MATERIALS AND METHODS

Study Area

The Pardinho River Basin is located in the central region of the Rio Grande do Sul State, in southern Brazil, and it has an area of 1,075 km^2 (Figure 1).

The Pardinho River is approximately 50 km long and is a tributary of the Pardo River. The Pardinho and Pardo rivers belong to the Jacuí River watershed, the largest freshwater basin of the Rio Grande do Sul State. The agricultural development in this region is based on small to medium sized properties and resembles the 18th century land tenure system in central Europe. Tobacco farming is the primary agricultural activity. The development of this region started with the arrival of German immigrants in the 19th century and became established with the introduction of tobacco companies at the beginning of the 1900s (Etges 1991).

The study of environmental pathways and human exposure to Mn in the tobacco farming area of Southern Brazil was performed using two steps. The research design consisted of a quantitative observational analytical transversal study.

The first step consisted of taking surface water samples from the Pardinho River to assess the physical-chemical characteristics and Mn levels of the water. Additionally, soil samples were taken from areas with and without agricultural activity. For the second step, a specific investigation was performed to assess the Mn exposure of the inhabitants in places where the first step showed high environmental levels of Mn. In the selected area, the criteria for choosing residents were the distance, and in some cases isolation, from an urban center, and those with their own food production systems, including vegetables, grains and animals, inhabiting drainage area and having children. Drinking water, food and hair samples were taken for analysis from 12 families (47 individuals ranging in age from 0.5 to 76 years). This number represents 5% of the total inhabitants of those areas with the highest manganese levels in the soil. This can be considered a finite population of the selected participating families and is enough to reach a confidence interval of 95% ($Z = 1.96$).

All the participants gave their permission to participate in the study (IRB approved informed consent) and were invited to answer a simple structured questionnaire designed to obtain information about age, food ingestion (home grown foods and foods acquired outside their properties) and the sources of the water they consumed. This project was registered in ethics committee number 1481/06.

Ground and Drinking Water Samples

Surface water samples from the Pardinho River were taken at four sampling sites along the river (upstream, middle 1, middle 2 and downstream), from May 2004 to May 2005 (autumn, winter, spring and summer).

Physical-chemical variables, such as pH, turbidity, chemical oxygen demand (COD), total phosphorus (P), Kjeldahl nitrogen (N), nitrate (NO_3^-), suspended solids (SS) and dissolved oxygen (DO) as well as suspended, dissolved and total manganese (Mn), were analyzed following the APHA (1998). A water quality assessment was made according to resolution number 357 of CONAMA 2005 (National Environment Council) for the classification of Brazilian continental freshwater.

Water samples (volume equal to 1.5 L) were collected using 0.5 L polypropylene bottles and preserved according to the analytical procedure as follows: without a preservation agent (pH, turbidity, NO_3^-, SS, and dissolved and suspended Mn), preserved with H_2SO_4 (P, N, and COD), and preserved with HNO_3 (total Mn). All samples were stored in coolers during transport to the laboratory.

In other selected areas, drinking water samples were collected to assess the exposure to Mn of the 12 families that live in the areas with the highest soil Mn content.

Soil Samples

In the first step, 64 sites were selected in the river basin, including areas with and without agricultural activity in the last few years (Figure 2). In areas with agricultural activity, soil was taken from tobacco fields (21 samples) and seedling production fields

Figure 1 - Location of the Pardinho River Basin, RS, Brazil.

(18 samples). In the second step, soil samples were taken where selected families lived (i.e., from their vegetable gardens and around the house). At each site, soil from at least 5 locations were randomly sampled from the upper horizon (0-20 cm) and combined to form a composite sample. A plastic bucket was used to mix subsamples, and a portion of approximately 0.5 kg was stored in a plastic bag. The samples were dried in a forced air oven at 45°C for 48 hours. The soil samples were then sized through a < 2 mm mesh sieve and analyzed for their total Mn content after digestion in PTFE (polytetrafluoroethylene) bombs with strong acids (HNO_3 and HF) at 120°C for 12 hours. After an extraction with KCl (1 mol.L^{-1}) for 5 minutes in a horizontal shaker, the solution was left standing overnight; subsequently, the bioavaliable Mn was measured. The Mn content was measured by flame atomic absorption spectrometry, fuelled by acetylene-air, Mn hollow cathode lamp (wavelength 279.5 nm, slit width 0.2 nm, 5 mA) with a double beam and a deuterium lamp for background correction (FAAS, Varian 55).

Food Samples

Available food crop samples, including cabbage (*Brassica oleracea– Acephala* and *Capitata* group), lettuce (*Lactuca sativa*), beetroot (*Beta vulgaris*), carrot (*Daucus carota*), radish (*Raphanus sativus*) and beans (*Phaseolus vulgaris*), were also collected from the sites where the selected families lived. At harvest, the plants were divided into shoots and roots and thoroughly washed with de-ionized water to remove all visible soil particles. After milling, samples were digested in PTFE bombs with strong acids (HNO_3 and HF) at 120°C for 12 hours. Manganese was measured using a graphite furnace atomic absorption spectrophotometer, applying 700°C and 2,400°C as the pyrolysis and atomization temperatures respectively, with a double beam and a deuterium lamp for background correction (GFAAS, Varian 55). Palladium was used as the chemical modifier.

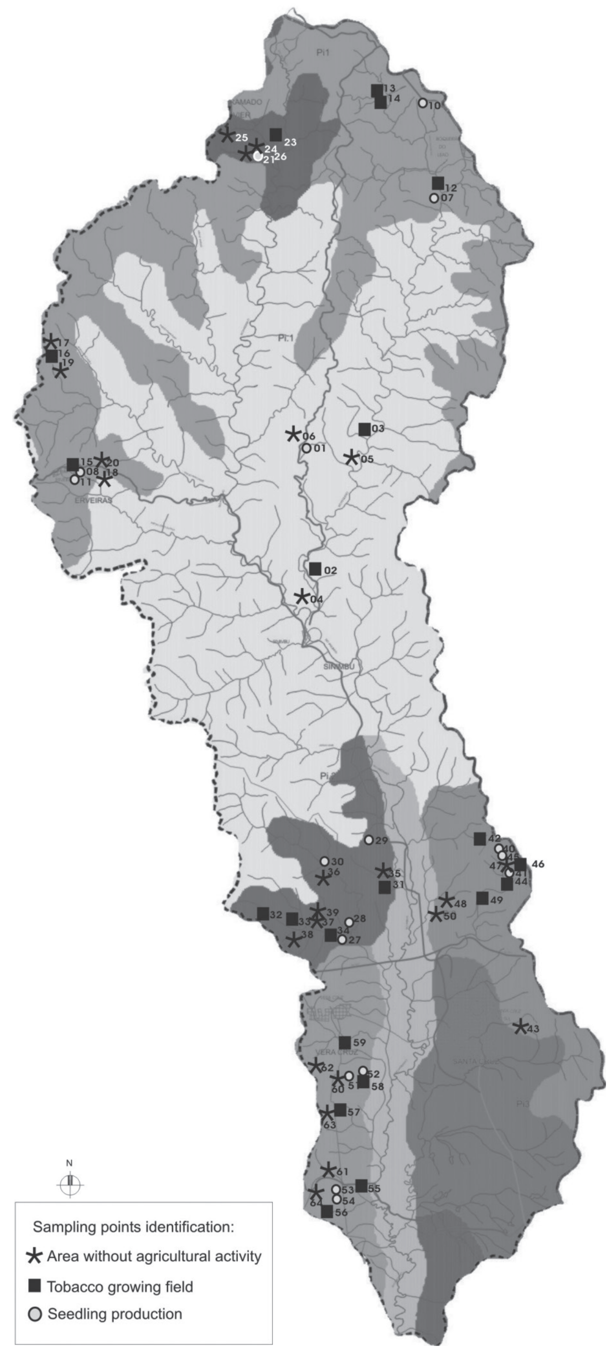

Figure 2 - Soil sampling points.

Hair Samples

Samples were collected using a pair of stainless-steel barber scissors. A small amount of hair was cut from the occipital region of the head and stored in a labeled paper envelope. The paper envelope containing the sample was then placed in a zip-lock polyethylene bag. Approximately 300 mg of each hair sample was washed with 20 mL of acetone p.a. for 10 min. After three short rinses with water to remove the organic solvent, the sample was washed three times for 10 min, each with 20 mL of a 1% solution of EXTRAN (MA-02, neutral, Merck). The concentration of the detergent and the number of repeated washings were chosen to provide the maximum removal of external contamination with a minimum loss of the endogenous elements (Miekeley et al. 1998); all washings were performed in an ultrasonic bath. After several final short rinses with Milli-Q water to remove the detergent, the samples were oven-dried at approximately 60°C overnight. The hair samples were digested in closed PTFE bombs and mineralized with concentrated nitric acid. The reagent and digestion conditions were chosen to achieve a complete mineralization and decomposition of the solid phase. After the acid digestion step, manganese levels were determined by means of a graphite furnace atomic absorption spectrophotometer, with a double beam and a deuterium lamp for background correction (GFAAS, Varian 55).

Statistical Analysis

All of the statistical analyses were performed using Graph Pad InStat® 3.00 software (ANOVA, T-test, Pearson correlation) and Pirouette® 2.7 software from Infometrix (Partial least squares regression – PLS). All tests were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Ground Water Samples

Table I shows the physical-chemical characteristics at 4 sampling points in the Pardinho River (upstream, middle 1, middle 2 and downstream) from 4 seasonal sampling dates.

The enrichment of contaminants along the river was primarily seen downstream at the point where the river receives wastewater discharges from Santa Cruz do Sul, the biggest city in the study region. The increase in contaminants is particularly noticeable with regard to turbidity, total nitrogen, phosphorus and suspended solids levels. At the middle points (2 and 3), no significant differences were observed, showing similar contributions of pollutants. Due to pollutant loads from Santa Cruz do Sul, the downstream sampling point showed an increase in levels compared to other sampling points ($P < 0.05$), with the exception of nitrogen, nitrates, and COD. The phosphorous levels (mean

TABLE I
Average of physical-chemical characteristics along the Pardinho river.

Sampling point	Turbidity (uT)	pH	N	P	NO_3	COD (mg.L^{-1})	SS	OD
Upstream	1.9	5.2	0.41	0.037	0.23	4.2	3.6	3.8
STD	2.7	0.2	0.35	0.048	0.05	3.8	2.8	
Middle 1	3.1	7.4	0.31	0.034	0.89	4.1	6.9	7.8
STD	1.5	0.2	0.22	0.012	0.70	2.5	2.8	
Middle 2	6.4	7.4	0.63	0.035	0.96	3.3	7.2	8.6
STD	0.7	0.3	0.72	0.008	0.78	2.4	2.3	
Downstream	22.5	7.2	1.28	0.187	1.05	7.3	23.4	10.4
STD	11.8	0.1	1.19	0.122	0.58	4.4	7.9	
P (Anova - linear tendency)	0.0006	< 0.0001	0.0987	0.0115	0.1000	0.2860	0.0001	-
P (Anova - among sampling points)	0.0015	0.0001	0.2673	0.0140	0.3444	0.3541	< 0.0001	-

ranging from 0.41 to 1.28 mg.L^{-1}) were above the environmental acceptable standard recommended by the Brazilian National Environment Council, which set a concentration of 0.1 mg.L^{-1} as the limit for this class of water (Resolution CONAMA n° 357). Other parameters did not exceed the limits recommended by the CONAMA.

Applying the analysis of variance (ANOVA) to test the significant differences of the linear tendency along the sampling points (upstream to downstream), significant differences were observed in turbidity ($P = 0.0006$), pH ($P < 0.0001$), phosphorous ($P = 0.0155$) and suspended solids ($P = 0.0001$). The dissolved oxygen levels did not indicate anoxic conditions at the sampling points of the Pardinho River.

Figure 3 shows the average values of dissolved, suspended and total Mn at the sampling points and their standard deviation. High variability in the downstream results was observed with significant increments of total Mn along the river ($P = 0.0042$).

The mean results of total Mn (113.3 µg.L^{-1}) in the downstream samples exceeded the limit set by resolution nº 357 of CONAMA (National Environment Council) for the classification of Brazilian continental freshwater (100 µg.L^{-1}), which is supplied for public consumption after simplified treatment. The European Communities (EC 1989) set that value in 50 µg.L^{-1}, but values up to 1,000 µg.L^{-1} in surface waters are accepted. Therefore, intensive systems of physical and chemical treatment may be necessary to adjust the quality of the public water supply (Homoncik et al. 2010, Marsden and Mackay 2001).

The dissolved Mn and suspended particle Mn levels showed a relative change along the river. Upstream, where the suspended solids content was low, most of the Mn was dissolved (63.9%). Downstream, most of the Mn was suspended (64%); a similar behavior was found in the Amazon River (83%), the Mississippi River (98%) and the Paraíba do Sul-Guandu River system (55%) (Malm et al. 1988).

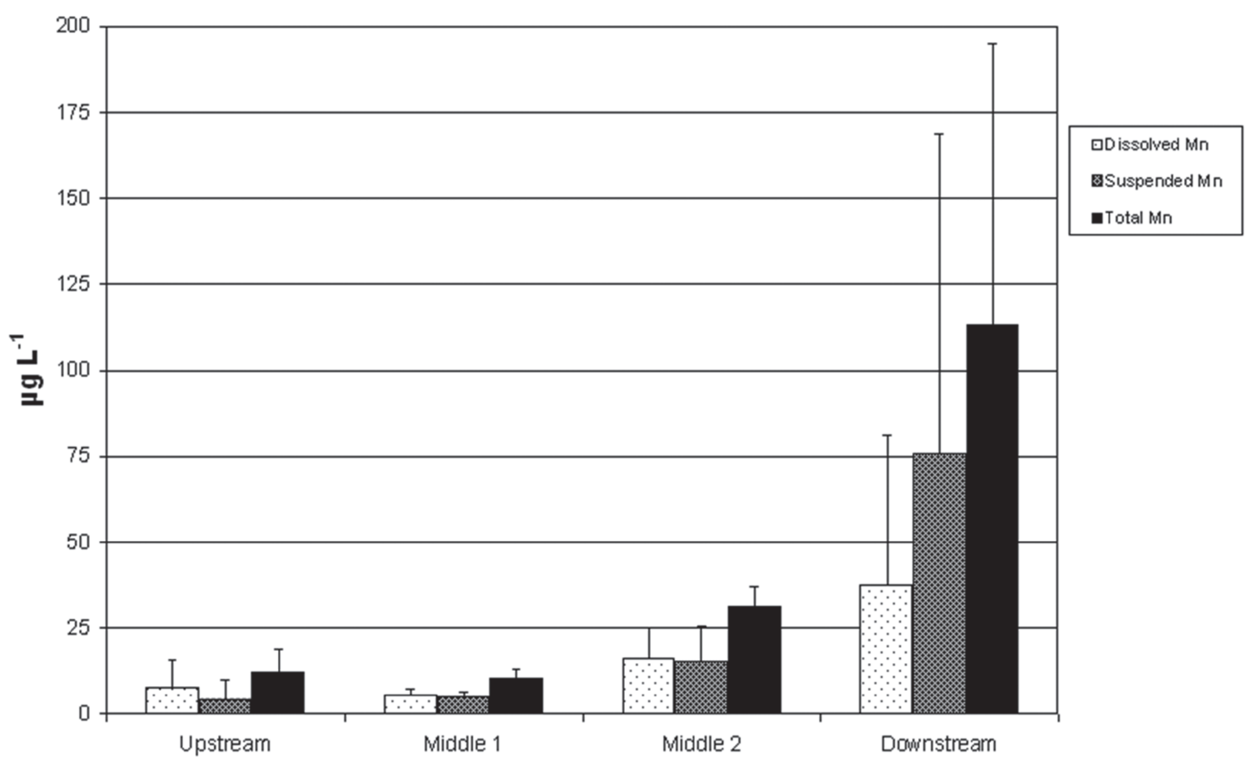

Figure 3 - Average and standard deviation of dissolved, suspended and total Mn in the Pardinho River.

The relationship of the dissolved and suspended Mn as well as the variations in this behavior was investigated through multivariate analysis, which considered the physical-chemical characteristics. A partial least squares regression (PLS) showed that there was no correlation between these values ($r = 0.24$, $P > 0.05$). Several combinations of independent variables were tested, and no correlations were found.

Soil

Table II summarizes the total Mn content from soils sampled in areas without agricultural practices, in the tobacco field and in seedling production areas.

TABLE II
Total Mn results of soil samples according to origin (without agricultural practices, Tobacco field and seedling production).

	Without agricultural practices (mg.kg^{-1}) (n=25)	***Tobacco field*** (mg.kg^{-1}) (n=21)	**Seedling production** (mg.kg^{-1}) (n=18)
Range	175.9 – 3,619.0	117.6 – 3,060.0	202.6 – 2,940.0
Average	1,235.9	1,136.9	883.1
STD	925.2	854.8	804.9

A T-test was applied to the averages and showed that there were no significant differences among the sampling areas (areas without agricultural practices vs. the tobacco field: $P = 0.710$; areas without agricultural practices vs. seedling production areas: $P = 0.200$). Thus, there was no Mn enrichment in soils affected by agricultural activity used in tobacco growing fields, even using mancozeb (according to farmers information) which contains Mn in its formulation (approximately 20%). High Mn levels appear to be due to natural factors (volcanic soil origin) according to what was previously described by Carvalho Filho et al. (2011), who stated that it was common to find birnessite (MnO) in the soil of Rio Grande do Sul State. There are possibilities that Mn level can be due to fertilizers use, but there is not a historic that in this region were used compounds that had manganese sulfate, manganese oxide or manganese chelate in the formulation.

Several sites showed Mn levels above the mean crust value of 900 mg.kg^{-1} (WHO 1981). Some of them, such as samples 3 and 5 (see Figure 2), were considered appropriate for the second part of this study. Families in that area are relatively isolated and distant from urban centers and produce their own food, including vegetables, grains and animals (i.e., little food was brought in). According to Bankovitch et al. (2003) and Rodríguez-Agudelo et al. (2006), the high Mn levels can be associated with particulate material inhalation, and therefore, the soil may be a contamination factor since manganese oxides in most of soils appears as finely dispersed particles (Carvalho Filho et al. 2011).

Families Selected for the Second Step

In the second step of the analysis to determine environmental exposure to Mn, 12 families were selected for the determination of Mn levels in their drinking water, food, and hair. Table III shows their characteristics, including age, vegetable production, animals used as food sources and food acquired outside their properties (data taken from questionnaire). The families identified as F8a, F8b and F8c belonged to the same family group and lived in close proximity, cultivating the same foods and using the same source of water. The locations of all families in the watershed are shown in Figure 4 and are in an area where high levels of Mn were found in soils in the first part of this study. Most of these families live along a tributary of the Pardinho River that runs between the mountains and contains rough paths that are difficult to access. This location provided a pattern of isolation influenced by local environmental conditions that led to the production and consumption of their own foods in a somewhat controlled environment.

TABLE III
Families' characteristics selected for the second step.

Family	Integrants (age)	Vegetable garden	Animals (as food source)	Foods acquired outside
F1	father (74); son (46)	cucumber, potato and lettuce	pig, hen and cow	Not asked
F2	grand father (76); father (43); mother (40); children (18, 6 and 2)	salads, parsley, turnip, broccoli and cabbage	pig, hen and cow	Cabbage, bean and rice
F3	father (54); mother (59); son (17)	salads, parsley, onion, cauliflower and cabbage	pig and hen	rice
F4	father (33); mother (30) son (12)	manioc, onion, potato, salads, cucumber, cauliflower, carrot and strawberrys	pig, hen and cow	rice
F5	father (39); mother (32); children (12 and 8)	cauliflower, beetroot, potato, bean and strawberry	pig, hen and cow	rice and fruits
F6	father (37); mother (30); children (12, 10)	salads, bean, potato and radish	pig, hen and cow	rice
F7	father (33); mother (28); daugther (2)	salads, cabbage, onion, carrot, cauliflower, radish and bean	pig, hen and cow	rice and potato
F8a	father (26); mother (16); daugther (5 months)			
F8b	father (45); mother (42)	salads, broccoli, carrot and cauliflower	pig, hen and cow	rice and bean
F8c	father (25); mother (23); children (4 e 6)			
F9	father (29); mother (25); son(3)	salads, cabbage, onion, carrot and cauliflower	pig, hen, lamb and cow	rice, bean, tea and sugar
F10	father (34); mother (26); son (2)	manioc, salads, potato and bean	pig, hen and cow	rice
F11	father (32); mother (28); children (6 and 4)	salads and bean	pig, hen and cow	rice
F12	father (32); mother (25); son (6)	bean, potato, salads	pig, hen and duck	rice

Environmental results from selected family locations

Drinking water and soil levels of Mn (total and the bioavailable fraction) were measured where the families lived. All families received drinking water from natural surface water sources, primarily coming from small creeks (springs). Three of the families had a well as their primary water source, and one family acquired water directly from the Pequeno River (tributary of Pardinho river), which was consumed after a single-step filtration (using a porous stone filter). Soil samples were taken where the vegetables were grown. Additionally, soil samples were taken from places near the homes where the children played, despite most houses having a lawn around them.

Table IV shows the results of the analyses of the drinking water and soil samples.

The drinking water results showed that there was no risk of exposure to Mn coming from this pathway. The Mn content was well below the acceptable limit established by WHO (0.1 $mg.L^{-1}$).

In soil samples, the total Mn contents were above the mean crust value, exceeding 2,000 $mg.kg^{-1}$ in 6 of the family locations (F2, F8, F9, F10 and F11). More important than the total Mn results were the elevated levels of bioavailable Mn.

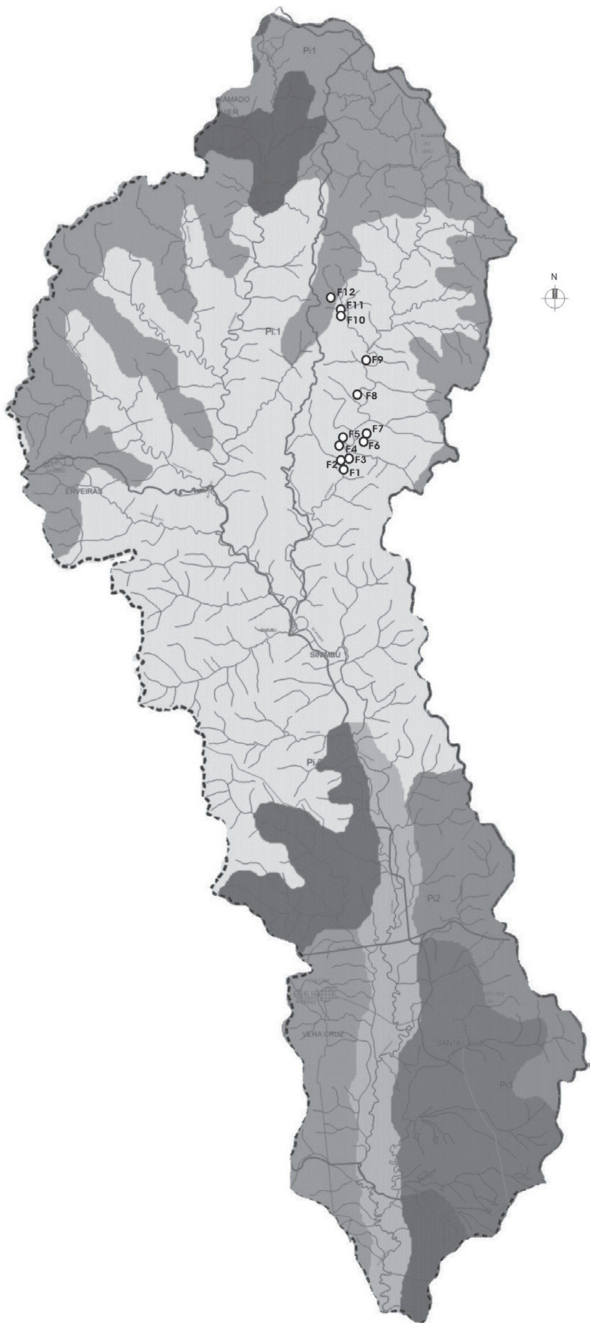

Figure 4 - Location of the selected family homes.

In agronomic terms, Mn levels above 5 mg.kg^{-1} are considered elevated (Tedesco et al. 2004). Most family locations showed high bioavailable Mn content, reaching up to 71.5 mg.kg^{-1}.

To verify that the high content of the bioavailable Mn in the solids would influence the levels of Mn in the food and become an important path for Mn exposure, the available vegetable and egg samples were analyzed. Table V presents the values found for the vegetables and eggs sampled.

Most of the food analyzed presented results near and above reference values. Among them, the samples that stood out were as follows: cabbage - Acephala group (all samples were above normal), cabbage - Capitata group (samples from F7, F8, F11 and F12 were above normal), lettuce (high content was found in samples from F2 and F8), beetroot (samples from F2 were 3 times higher than normal), carrots (all samples were above normal) and beans (both samples collected, from F6 and F10, were quite high, approximately 8 and 5 times higher than normal, respectively). Despite the high content of Mn in vegetables, no correlations were found with the Mn in soil. Only the Mn in carrots showed a significantly positive correlation with the total Mn in the soil, but not with the bioavailable Mn. Because few families produced beetroots, radishes and beans, no correlation could be made with these items. Though few, the results found in bean samples were well above the reference value (ranging from 48.2 to 81.8 mg.kg^{-1}, 4 and 8 times the reference value [10.6 mg.kg^{-1}], respectively) (USDA 2008). Families F6 and F10 cultivated beans in soils with high levels of bioavailable Mn. Due to its importance in the diet (staple diet in the South of Brazil), beans could be considered an important path of oral exposure to Mn, and special attention should be given to this food. The results showed that further investigations need to be performed in which the number of samples (including rice, potatoes, flour, meat, among others) is increased and the daily intake of Mn from foods in the studied region is assessed.

Hair samples from selected families

The average, minimum and maximum concentrations of Mn in hair found in family integrants, as well as the standard deviation values are shown in Table VI. When the sampling was performed,

TABLE IV
Mn content in drinking water and soil samples taken from selected families' places.

Family	Drinking water source	Drinking water Mn ($mg.L^{-1}$)	Total Mn (vegetable garden soil) ($mg.kg^{-1}$)	Bioavailable Mn (vegetable garden soil) ($mg.kg^{-1}$)	Total Mn in soil around house ($mg.kg^{-1}$)	Bioavailable Mn in soil around house ($mg.kg^{-1}$)
F1	spring	0.004	1,515.0	35.0	-	-
F2	well	0.001	2,641.6	47.3	-	-
F3	well	< 0.001	-	-	-	-
F4	spring	0.001	1,301.8	30.9	-	-
F5	spring	0.001	1,491.0	29.1	-	-
F6	spring	0.003	1,622.0	71.5	-	-
F7	spring	0.002	1,858.8	1.4	199.6	4.0
F8a						
F8b	spring	0.008	2,447.8	0.9	861.2	52.4
F8c						
F9	spring	0.001	2,076.2	0.3	-	-
F10	well	0.001	2,433.0	36.9	-	-
F11	spring	< 0.001	1,320.6	56.0	-	-
F12	river	0.003	775.3	23.0	-	-

TABLE V
Mn content in foods collected in selected families' places.

Food	Reference value*	Family												Correlation coefficients (r) with Mn soil content	
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	Available Mn	Total Mn
Egg white	0.11	0.21	0.14	-	0.47	0.18	0.15	0.14	0.15	0.15	0.15	0.15	0.14	-	-
Yolk	0.55	0.46	0.52	-	0.41	0.49	0.25	0.36	0.61	0.36	0.65	0.40	0.40	-	-
Cabbage (*Brassica oleracea - Acephala* Group)	2.76	10.5	7.08	-	5.08	25.1	5.54	-	7.32	15.1	8.60	7.44	-	-0.2626 (P=0.4335)	-0.1598 (P=0.6909)
Cabbage (*Brassica oleracea - Capitata* Group	1.59	1.75	0.58	-	0.51	-	0.43	19.5	12.8	-	-	8.61	3.78	-0.4907 (P=0.1499)	0.0264 (P=0.9422)
Lettuce (*Lactuca sativa*)	2.50	-	15.6	-	-	-	1.66	2.72	26.7	3.02	1.89	1.39	1.42	-0.3023 (P=0.4667)	0.5962 (P=0.1187)
Beetroot (*Beta vulgaris*)	3.29	-	19.2	-	1.62	-	-	-	-	-	-	-	-		
Carrot (*Daucus carota*)	1.43	-	5.56	-	3.19	3.90	2.47	3.70	6.35	5.16	-	-	-	-0.4123 (P=0.3580)	0.8590 (P=0.0133)
Radish (*Raphanus sativus*)	0.69	-	-	-	0.50	-	-	0.48	0.63	-	-	-	-	-	-
Bean (*Phaseolus vulgaris*)	10.6	-	-	-	-	-	81.8	-	-	-	48.2	-	-	-	-

** Reference value according to USDA National Nutrient Database for Standard Reference, Release 21 (2008).*

no integrant of family F3 was at home, and only one subject was at home in family F11. To compare results, Table VI also shows reference values and values taken from other studies.

Compared to the reference (maximum of 0.75 mg.kg^{-1}, from the non-exposed population), high values were found in hair from most of the studied subjects (ranging from 0.17 to 39.59 mg.kg^{-1}, mean 5.32 ± 9.03 mg.kg^{-1}). Only one family (F7) did not present an average above the reference level. Approximately 75% of the results from all samples were above the maximum limit, reaching high values (10 to 40 mg.kg^{-1}) for 7 integrants (17%) that belonged to families F2, F4, F6 and F8b.

TABLE VI
Human hair levels of Mn in family integrants.

Family	Number of integrants	Age range (years)	Mn range (mg.kg^{-1})	Mean and standard deviation (mg.kg^{-1})	Reference values*
F1	2	46 - 74	2.56 – 8.11	5.34 ± 3.92	Reference: 0.26 – 0.75
F2	6	2 - 76	0.21 – 29.75	9.49 ± 11.74	
F4	3	12 - 35	5.48 – 10.91	7.33 ± 3.10	Wroclaw: 0.601
F5	4	1 - 38	0.83 – 7.06	3.94 ± 2.64	
F6	5	10 - 59	0.30 – 33.31	7.24 ± 14.58	South Poland: 2.41
F7	3	2 - 33	0.24 – 0.72	0.48 ± 0.24	
F8a	3	0.5 - 26	0.52 – 3.55	2.15 ± 1.53	Sweden: 0.560
F8b	2	42 - 45	1.21 – 39.59	20.40 ± 27.14	
F8c	4	3 - 28	1.10 – 1.52	1.59 ± 0.54	Rio de Janeiro: 0.1 – 1.8
F9	3	4 - 30	0.17 – 0.96	0.84 ± 0.62	
F10	3	2 - 34	0.55 – 1.26	0.91 ± 0.36	
F11	1	32	-	11.22	
F12	2	6 - 25	1.20 – 3.77	2.49 ± 1.82	
Total	**41**	**1 - 76**	**0.17 – 39.59**	**5.32 ± 9.03**	

*Reference values for human hair (Miekeley et al. 1998), Mn concentrations (mg.kg^{-1}) in hair for 83 subjects living in an urban area in south west Poland (Wroclaw city) (Chojnaca et al. 2006), for 266 subjects living in a non-industrialized region of south Poland (Silesian Beskid Slaski) (Nowak 1998), for 114 subjects from a urban population group living in north-east Sweden (Rodushkin and Axelssom 2000) and for 1091 subjects of Rio de Janeiro City (Miekeley et al. 1998).

The Mn content in the hair of the studied subjects was also compared to an urban area in southwest Poland (Wroclaw) and populations living in non-industrialized areas of Poland (Silesian Beskid), Brazil (Rio de Janeiro) and Sweden (northeast). Average values obtained from families F1, F2, F4, F5, F6 and F8b (mean ranging from 3.94 to 20.4 mg.kg^{-1}) were above the values of the reference studies (mean ranging from 0.1 to 2.41 mg.kg^{-1}).

The results indicated a high intake of Mn by the selected family subjects. No significant correlations were found with age or soil levels ($P > 0.05$). However, most soil samples showed high contents (derived from Volcanic action), which could explain the high levels found in analyzed foods, the probable source of Mn. Because some studies have suggested that ingestion of foodstuffs containing increased concentrations of manganese may result in adverse neurological effects (Kawamura et al. 1941, Kilburn 1987, Kondakis et al. 1989, Goldsmith et al. 1990, He et al. 1994, Iwami et al. 1994, Zangh et al. 1995), the study area needs to be further investigated, with an assessment of the actual daily intake of Mn (increase sample numbers and analysis of other types of important daily foods, such as meat, flour, rice, among others) and testing of the prevalence of neurological effects of the people in these areas to assess if these exposure levels can adversely affect the health of the inhabitants of the Pardinho River Basin.

CONCLUSION

An environmental assessment was taken along the Pardinho River; a significant increase in Mn levels was found, which were above the environmentally acceptable standards recommended by the Brazilian National Environment Council (CONAMA). In addition, there was no direct relationship to anthropogenic activities because the results of the soil analysis did not indicate significant differences in Mn levels between samples from areas without agricultural activity vs. areas with seedling production or tobacco fields.

Drinking water results showed that there was no risk of exposure to Mn coming from this source. The soil samples from the families' properties showed levels above the crust mean value (ranging from 775.3 to 2,641.6 $mg.kg^{-1}$). Bioavailable Mn results were also high, reaching 71.5 $mg.kg^{-1}$, which was significantly above levels required for agronomical purposes. The soil has been recognized as the predominant factor of risk exposure, and this problem was reflected as a source of bioaccumulation in humans because Mn levels from hair samples of most of the subjects studied were high. Although the contamination seemed to have a natural origin, the results found in the present study showed that Mn levels in the Pardinho River Basin are a relevant public health issue that needs to be deeply investigated. The sampling design performed in this study showed that all of the studied family groups were exposed to excess Mn. To assess if these exposure levels can adversely affect the health of the living inhabitants of the Pardinho River Basin, a further study must be conducted to improve the sampling design, assess the actual daily intake of Mn and to test the prevalence of neurological effects associated with oral exposure.

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RESUMO

Neste estudo Da exposição humana e ambiental ao Manganês (Mn) no Sul do Brasil foi realizada em duas etapas. O primeiro passo consistiu na análise de amostras de águas da superfície do rio Pardinho. Os resultados médios desta tecnica mostraram aumento significativo de poluentes, incluindo aumento dos níveis de Mn acima do padrão aceitável para o ambiente recomendado pelo Conselho Nacional de Meio Ambiente. Além disso, 64 amostras de solo foram coletadas em áreas com e sem atividade agrícola. Muitos resultados ficaram acima da média e não indicaram diferenças significativas dos níveis de Mn entre as áreas amostradas. Para o segundo passo, 12 famílias foram selecionadas e avaliadas quanto à exposição ao Manganês nas regiões com níveis elevados de Mn no solo. A maioria dos alimentos analisados continham Mn acima dos valores de referência, indicando que o alimento pode ser uma fonte importante de exposição. O teor de Mn no cabelo da maioria dos indivíduos estudados foi também elevado em comparação com valores de referência de populações não-expostas. Apesar de a contaminação parecer vir de uma origem natural, os resultados encontrados neste estudo mostraram que os níveis de Mn presentes na Bacia do Rio Pardinho são um relevante problema de saúde pública.

Palavras-chave: manganês, exposição humana, níveis ambientais, Mn.

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