

**LEVEL OF EXPOSURE TO LEAD ELIMINATED BY AUTOMOBILES –
IMPACT ON OXIDATIVE STRESS AND THE NUTRITIONAL STATUS OF
SENEGALESE CHILDREN.**

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INTRODUCTION

The use of automobiles has considerably increased throughout the world. However, over the last few decades the growth rate has slowed down in highly industrialized countries, whereas demographic growth and urban and industrial development have accelerated the use of motorized vehicles namely in developing countries.

Thus, in Senegal like in many of these countries, a rapid uncontrolled growth of the automobile fleet can be seen and could be the origin of negative effects on health particularly in cities.

Several studies have shown the clear relationship between automobile traffic density, the use of leaded gasoline and lead content in the air and soil in urban zones (1,3,4,11,15). Studies carried out in Dakar show that lead content in the barks of trees bordering the highways correlates to traffic intensity (6). These results therefore show that the use of leaded gas is the main cause of air contamination and could be one of the principal sources of exposure to the public especially in children who constitute the most vulnerable group.

Adverse effects of Pb on neurobehavioral development in children are observed even with low blood concentrations. Children can also develop symptoms of extreme intoxication with encephalopathy, which is usually irreversible at blood lead levels exceeding 800µg/l (13). To prevent these negative effects, screening of pre-school children was recommended by the American Centers for Illness Control and the American Pediatrics Association. Then, the Organizations of United Nations (PNUE and UNICEF) and the European Union recognized that exposure to lead present in the atmosphere is a major threat to health and called for appropriate actions to be taken to reduce blood lead levels to below 100µg/l and to eliminate this metal in gasoline (25).

This problem however is not really known in Senegal where data on population exposure is not available.

These considerations brought us to carry out this study on Senegalese children in order to:

- Measure the level of impregnation of lead eliminated by vehicle exhaust
- Study the biological levels of precocious markers
- Study the possible relationship between blood lead level and oxidative stress markers
- Evaluate the nutritional status of children who have been exposed to lead from Dakar's traffic.

11. METHODOLOGY

Study population

This study was carried out in West Africa, in Senegal. It is a transversal epidemiological study comparing two populations, the exposed children living in urban areas (Dakar) and the non-exposed children living in rural areas (several villages in Khombole in the region of Thiès).

The participating children were selected at random, any child whose parents gave their consent was chosen. Some criteria however were respected:

- Inclusion: children had to be between the ages of 8 and 12, and been living in the study zone since their birth
- Exclusion: all children with a known pathological condition were excluded

The study began on February 26th 1999 and ended on August 19th 1999.

A questionnaire was given to the families of participating children to try and determine exposure sources.

Blood samples were drawn from the veins by EDTA, and urine samples were taken. The samples were conserved by freezing, first at -20°C and then at -80°C.

Biological determinations

Lead was dosed by Atomic Absorption Spectrometry (Perkin Elmer 5100 Z AS-60) using a graphite oven with Zeeman correction. The limit of detection was 0.10µg/dl with a precision of 0.015µg/dl. Repeated analyses of standard solutions enabled the method's precision to be confirmed. Obtained results were expressed in µg/L.

Concentrations of ProtoporphyrineZinc (PPZ) were measured by fluorimetry (excitation 415nm, emission 595nm) in the total blood.

Urinary concentrations of ALA were measured by spectrophotometry ($\lambda=546\text{nm}$).

Determinations for oxidative stress markers are specified for each parameter: GST (urine): Enzyme Immunoassay test (ELISA); MDA (plasma); HPLC separation UV detection; GR, GPX, SOD (total blood) Fara Cobas II; GSH, GSSG (total blood); HPLC electrochemical detection.

Evaluation of the nutritional status

Measuring weight:

Children's weight was measured with an electronic scale OHAUS (1-10 Model IS 100 A, Germany) maximum weight of 150kg and a precision of 50g.

Measuring height:

Children's height was measured with a wooden measure and tape with a precision of 0.1cm.

The indexes for weight according to age P (A) and weight according to height P (T) were calculated following references from the National Center for Statistics and Health (NCHS) adopted by WHO as the international basis. The EpiNut programme from Epi Info software 6.0 version allowed the indexes to be calculated and translated into Z scores. Allix indexes lower than -2 Z score were considered to be sub-normal.

Data analysis

EpiInfo software, version 6 (The division of Surveillance and Epidemiology – Epidemiology Program – Office Centers for Disease Control and Prevention) was used for statistical analysis.

Averages and standard deviations were calculated for each variable and variations amongst the different groups were analyzed utilizing the variant analysis (ANOVA) and the student test. Differences are considered to be significant when the $p<0.05$.

III. RESULTS AND DISCUSSIONS

In relation to the impregnation parameter: blood lead level (figure 1), a significant difference was observed ($p=0$) between blood lead levels observed in children living in urban areas (average $106.6 \mu\text{g/l}$) and those living in rural areas (average $68.1 \mu\text{g/l}$). This is the maximum level in a child tolerated by the CDC since 1991.

It is important to note that these high levels of blood lead seem to be caused by lead from automobiles. No industries likely to expulse lead into the atmosphere exist within our study zone.

In spite of the absence of data on possible contamination from lead in paint, we believe this source to be slight, given the types of habitat especially in rural areas. Some lead piping could still exist in Senegal resulting in water contamination, but after questioning

the various households, they did not have them and this source of contamination does not exist in rural areas.

If the results are compared with those from similar studies carried out in Africa, the values are quite similar. NRIAGU J., 1997 carried out two studies; the first one was in Nigeria, in the medium sized town of Kaduna and the average blood lead level in children aged between 1 and 6 years old was 106 µg/l. In the second study, in South Africa, covering 1200 children aged between 3 and 10 years old, the average blood lead level of children living in urban areas was 100µg/l and 38µg/l for those living in rural areas (NRIAGU, OLERU et al., 1997a. NRIAGU, JINABHAI et al., 1997b). It must be noted that the levels found in our study are very high compared to those of children living in industrialized countries. In the USA children between the ages of 1 and 6 yrs had levels of 27µg/l from 1991 to 1994, whereas these were 40µg/l in 1988 to 1991, and approximately 150µg/l from 1976 to 1980 (13). In France, the average blood lead levels were estimated at 36µg/l in 1995 in children between 1 and 6, expecting to reach 25µg/dl by 2000 (14). The reduction of average levels of blood lead in industrialized countries since the 80s is linked to the implementation of the “unleaded” policy that began earlier in the States than in France.

It is also interesting to compare the blood lead levels obtained in boys and girls, where a significant difference is noted ($p < 0.005$): boys have higher blood lead levels than girls, an average of 98.00 µg/l in boys compared to 78.90µg/l in girls. If averages are calculated according to regions and gender, boys living in Dakar have the highest, the average being 120µg/l. This difference can be explained by the fact that boys are more exposed than girls as they spend their time playing outdoors. It has also been observed that blood lead levels are higher in men than in women (12). After analysis of variables showing that blood lead levels vary significantly according to gender and exposure, it can be said that gender represents a notable factor.

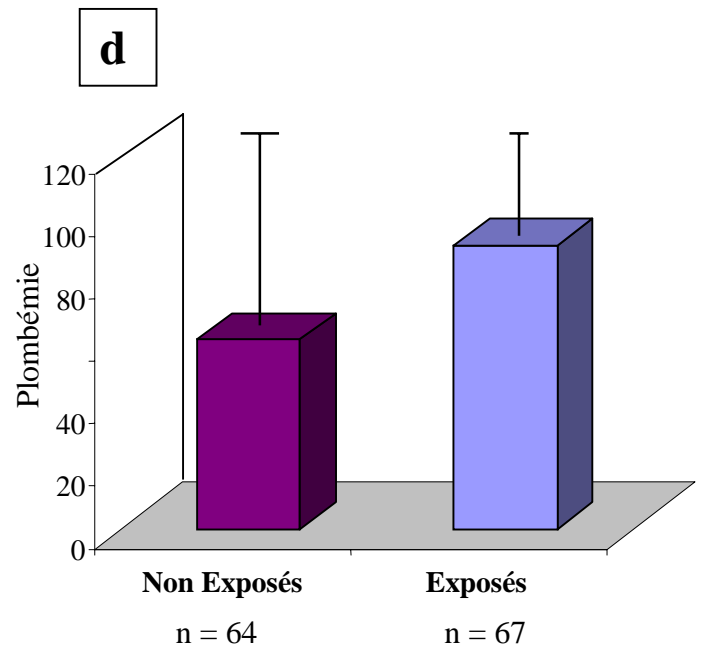
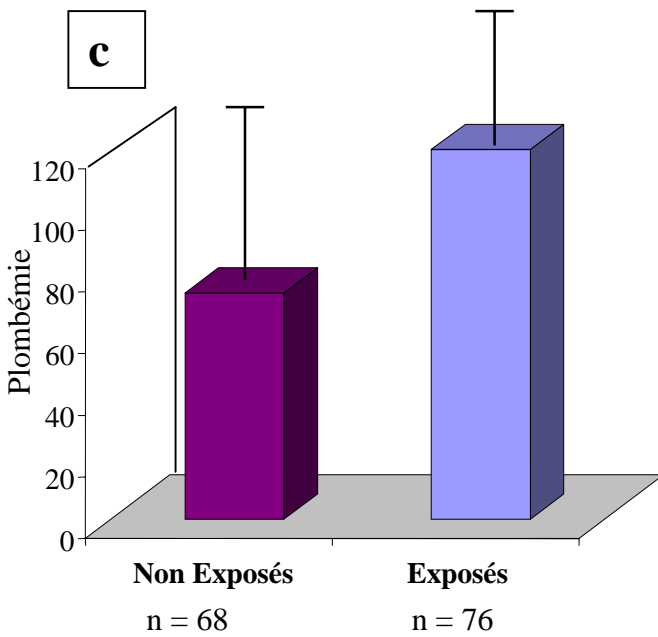
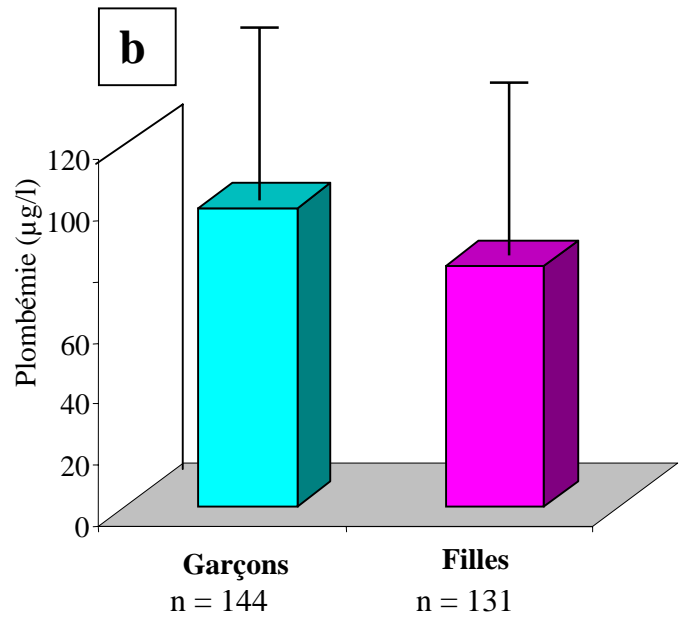
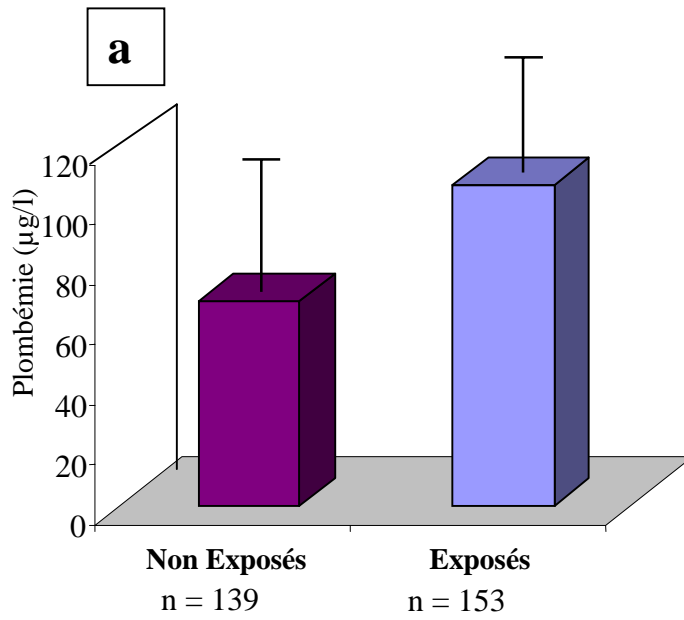


Figure 1 : Plombémies (moyenne et écart-type)
a : en population totale non exposée et exposée
b : chez les garçons et chez les filles
c : chez les garçons non exposés et exposés
d : chez les filles non exposées et exposées

In a similar study, exposure of children to lead, evaluated by the blood lead level, was related directly to concentrations found in dust and soil, and directly related to the size of the town the children lived in and the density of the traffic (17,18). Furthermore, in several studies, the diminution of leaded gas utilization was immediately correlated to a diminution in average levels of blood lead (20).

For the effects of synthesis on the heme, the average ALA from urine obtained from the total population (n=292) is 5.31mg/g of creatine \pm 4.58. In non-exposed children (n= 139) an average of 4.77mg/g of creatine \pm 4.24 whereas in exposed children (n=153), the average reaches 5.80mg/g of creatine plus \pm 4.80. It should be noted that level dispersion appears to be of importance when the typical gaps are as big as the averages.

According to the non-parametric test U of Mann-Whitney, the difference between the two populations is significant ($p < 0.001$). A significant difference is seen ($p = 0$) when comparing levels of urinary ALA of exposed children to non-exposed children. Furthermore, the correlation between blood lead levels and urinary ALA on the whole population is good and positive ($p = 0$). ALA-dehydratase (ALA-D), that catalyzes the two ALA molecules to give uroporphyrinogen, is known to be the most sensitive enzyme to lead, the inhibition of this enzyme results in an accumulation of ALA in the plasma and its urinary excretion (13).

Recent studies have suggested that damages caused by lead could have partially at their origin oxidative stress due to ALA which, accumulating, could generate the formation of the peroxide ion (14). In NEAL's study (1997), administration of ALA in vitro to hamster cells CHO (Chinese Hamster Ovary) showed a reduction of GSH parallel to an increase of GSSG, thus suggesting the existence of oxidative stress.

Urinary ALA therefore seems to be a good marker for evaluating lead exposure. However, given inter-individual susceptibility and possible fluctuations (in the beginning or at the end of lead exposure), urinary ALA is not always considered to be an adequate evaluation measure of lead toxicity.

Concerning PPZ, the average PPZ erythrocytaries obtained from the total population (n=281) is of 3.60 μ g/g of hemoglobin \pm 1.66; this average is of 3.71 μ g/g of hemoglobin \pm 1.96 in non-exposed children (n=130) and 3.50 μ g/g of hemoglobin \pm 1.35 in exposed children (n=151). For PPZ, the non parametric U test of Mann-Whitney did not show a significant difference between the two populations ($p = 0.617$), a significant difference was not observed between the two populations, exposed and non-exposed ($p = 0.617$), furthermore blood lead levels do not correlate to PPZ levels. These results, however, are not surprising, as PPZ only increases in blood to important blood lead levels (approx. 250 μ g/l). With the present blood lead level threshold for children of 100 μ g/l the predicted level of PPZ is not sufficient to screen children who are at risk; the measure of erythrocytary porphyrins would not be enough to detect blood lead levels below 100 μ g/l (MARKOWITZ et al, 1997). Furthermore, the sensitivity of the dosage will quickly diminish when blood lead levels are less than 450 μ g/l and will become inferior to 50% for blood lead levels less than 200 μ g/l (2).

PPZ dosage is therefore not a good indicator of weak lead exposure (HEINZE et al, 1998). This dosage is also not considered to be specific to infant saturnism, particularly because of risk of anemia in children (PPZ increases during iron deprived anemia) (2).

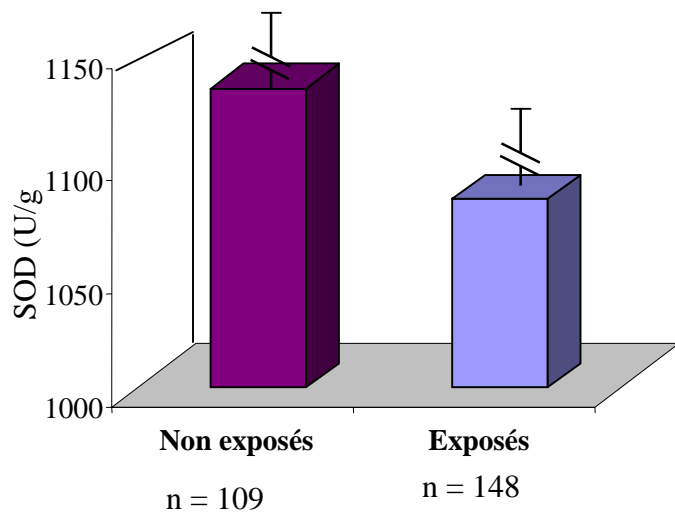


Figure 2 : Activité de la SOD chez les non exposés et les exposés

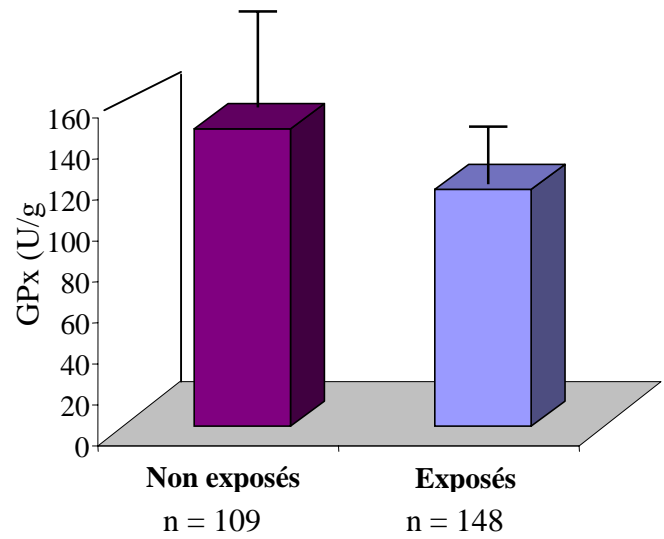


Figure 3 : Activité de la GPx chez les non exposés et les exposés

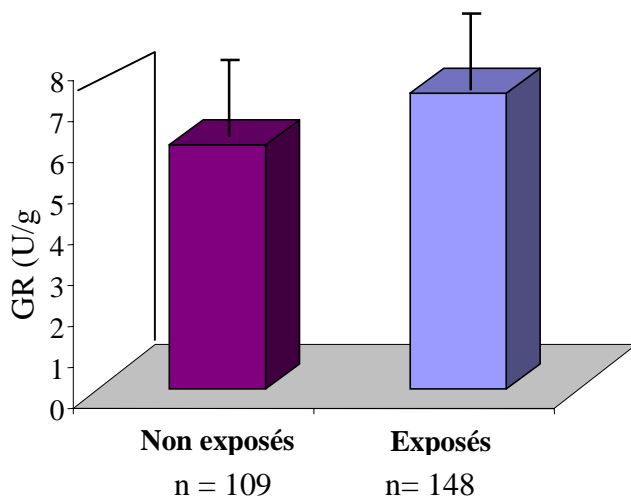


Figure 4 : Activité de la GR chez les non exposés et les exposés

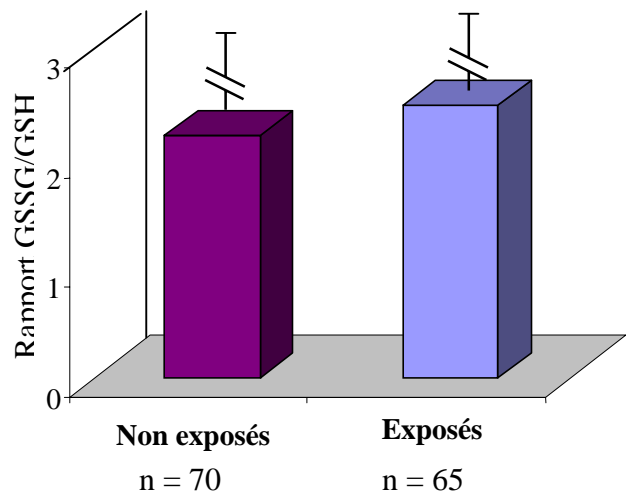


Figure 5 : Rapport GSSG/GSH chez les non exposés et les exposés

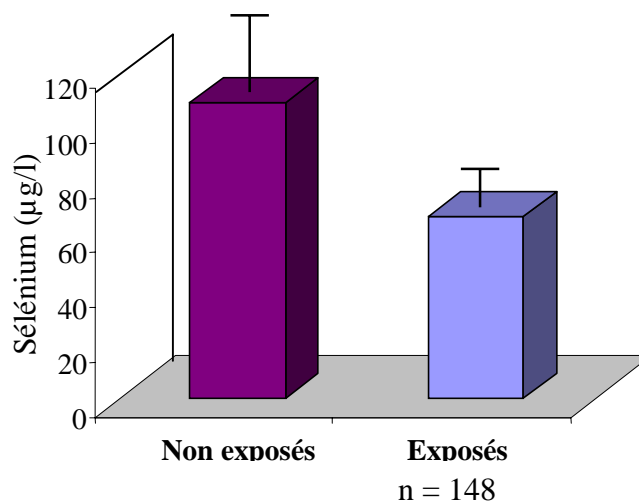


Figure 6 : Taux de sélénium chez les non exposés et les exposés

Measuring effects of oxidative stress

Correlations between blood lead levels and different parameters

Using Spearman's regression, we determined correlations between blood lead levels and studied parameters, whose thresholds of correlation signification are shown in Table 1V.

	ALA	PPZ	SOD	GPx	GR	Se	GSSG/GSH
Blood lead level	*** p<0,001	NS p = 0,380	NS p = 0,215	*** p<0,001	NS p = 0,143	*** p<0,001	NS p = 0,870

Table I : Correlation between blood lead levels and studied parameters

*** : significative, p<0,001

** : significative, p<0,01

* : significative, p<0,05

NS : non significative, p>0,05

The corresponding correlation angles are represented in figure 10, they were drawn up using log values and give a coefficient of correlation of r2.

Correlation between certain parameters and oxidative stress

We also determined correlations between different parameters of oxidative stress whose thresholds are given in table V

	GR	Se	GSSG/GSH
GPx	NS p = 0,556	*** p = 0	NS p = 0,268
GR		** p = 0,002	NS p = 0,830

Table II : Correlations between GPx, GR, selenium and the relationship GSSG/GSH

Reduced and oxidized glutathion

The relationship GSSG/GSH was determined, the average relationship obtained from non-exposed children (n=70) is of 2.21 ± 3.11 whereas that of exposed children (n=65) is of 2.49 ± 5.38 (Figure 5). The relationship GSSG/GSH is therefore higher in the exposed population and typical gaps are also high.

The GSSG?GSH relationship is higher in exposed individuals, with a significant difference (p=0.043); this implies an increase in GSSH level and a reduction of GSH.

This result corroborates the hypothesis that in the case of oxidative stress, GSH is consumed in excessive fashion to fight against the apparition of free radicals (O_2^- , H_2O_2), GSSG is thus formed in large quantity by the oxidation of GSH by the action of GPx. Furthermore, it has already been shown that lead is responsible for the diminution of GSH as well as thiol groupings (-SH). (11).

Anti-oxidant enzymes and selenium

Different enzymatic activities averages in the two populations were represented on histograms.

The average obtained for SOD activity is $1132.65U/g$ of Hb ± 232.57 in the non-exposed (n=109) and $1083.62 U/g$ of Hb ± 230.62 in the exposed (n=148) (figure 2).

A decrease in enzymatic activity was also noted in the exposed population, the averages being $145.06 U/g$ of Hb ± 56.50 , in the non-exposed (n=109) and $115.95 U/g$ of Hb ± 38.53 in the exposed (n=148) (Figure 3).

On the other hand, for GR, enzyme activity is higher in the exposed population (n=148) or $7.20U/g$ of Hb ± 2.54 as opposed to 5.96 ± 2.25 in the non-exposed (n=109) Figure 4.

The average serum selenium observed in non-exposed children is (n=132) is $108.13 \mu g/l \pm 33.54$ and is much weaker in exposed children (n=148), or $66.57 \mu g/l \pm 18.58$ (figure 6).

➤ SOD

The slight diminution, non significant ($p=0.076$) of SOD activity in exposed children is explained by an important consummation of this enzyme in order to fight against the increased production of O_2^\bullet during an oxidative stress phenomenon. This corroborates most of the data showing that anti-oxidant enzyme activity (SOD, catalase, GPx, GR) was experimentally inhibited by lead exposure (11). An increase in SOD activity (Costa et al 1991) was also noted after lead exposure.

Furthermore, no correlation has been noted between SOD enzyme activity and blood lead levels. Therefore, in this study, activity variations of this enzyme cannot be correctly ascertained, because of the non-significant fact results.

➤ GPx

An increase in GPx activity was observed, significantly less in exposed individuals ($p=0$). As for SOF, this also seems to accompany an increased consummation of the enzyme in order to de-toxify H_2O_2 produced from O_2^\bullet - in the event of intense oxidative stress.

GPx needs GSH as a co-factor, the latter being weaker in exposed individuals (given the higher relationship), the enzyme cannot therefore function normally. Also, as the

selenium level is significantly lower in exposed children ($p=0$), reduced GPx activity is logical, as selenium is another indispensable co-factor to this enzyme.

A significant correlation between blood lead level and GPx also exists as it does with blood lead level and selenium. Reduced GPx and selenium level activity is therefore related to blood lead level increase. These two parameters, GPx and selenium, are therefore interesting markers of lead effect on oxidative stress.

➤ GR

Contrary to the inhibition observed for GPX activity, significantly higher activity was observed in exposed children than for GR ($p=0$). It was shown that in individuals who were exposed professionally to lead, GR was higher, suggesting the presence of a compensating mechanism to thiol group losses (-SH) that are normally brought by GSH and are responsible for anti-oxidant activity (11). GR seems to be solicited at this stage by oxidative stress to regenerate GSH whose level is reduced in children exposed to lead.

Finally, a most significant correlation is noted between GPx and its co-factor, selenium, which confirms the hypothesis that we formulated concerning these two parameters. Furthermore, a correlation between selenium and GR exists whose activity is coupled with that of GPx.

This obtained prevalence shows that protein energetic malnutrition is a phenomenon that is cut off in this age group. In relation to previous studies carried out on children aged between 0 and 5, (6), the nutritional situation in urban and outlying zones continues to worsen. A recent study carried out in schools showed levels that are comparable to our studies' with a deficit of 10.4 % and 5.0 % of late growth in school children (21) as opposed to, respectively, 11% and 6% in our study. Our study confirms the results from schools. We calculated the relative risk (RR) of being malnourished for children exposed to lead. The test is significant for children suffering from ponderal deficit (RR=0.34; to 95%, $p=0.92$) whereas the probabilities are higher than 0.05 for other types of malnutrition found in the children studied.

	Late growth T(A)	Ponderal Deficit P(A)	Emaciation P(T)
Boys (85)	8	18	18
Girls (88)	3	4.5	12.5
Total (173)	6	11	36

Table III: Malnutrition prevalence

The highest proportion of anemic children (63.9%) is found in the group whose blood lead levels are higher than 100µg/l. Lead affects the hemoglobin level ($p=0.01$). In the same way, contaminated girls are more anemic than those whose blood lead levels are less than 100µg/l. ($p=0.03$). The coefficient correlation calculation ($r=-0.047$; $p=0.05$) between lead and hemoglobin has shown that blood lead levels have a negative effect on hemoglobin level in children, when the blood lead level increases, the hemoglobin level diminishes.

The influence of anemia and iron deficiency was evaluated in children and 54% of the children are anemic, with 17.4% of the children suffering from iron deficiency. These results are lower than the prevalence of anemia in school age children, which is estimated at 49.8% in Africa (25). They are also far superior to those found in studies relating to anemia in schools, which is 33.46% (21). Blood lead level is probably conducive to anemia in the children in our study. Here, blood lead levels influence the nutritional status through anemia, especially in a martial context. Iron deficiency can cause poor academic performance due to a decline in cognitive development, from attention to concentration ability (26). It can thus increase susceptibility to saturnine impregnation by being conducive to lead fixing on red blood cells.

This study will have allowed the sources of exposure to lead to be better evaluated in Senegal. We have been able to show that through the blood lead levels obtained, an important fraction of Senegalese children have been impregnated. This confirms the presence of an important public health problem that could be certainly controlled by the implementation of regulations designed to lower lead levels in gasoline.

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