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Resistance to Lead of *Streptococcus mutans* Isolated from Adolescents in San Baltazar Tetela, Puebla

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Authors' contributions

This work was carried out in collaboration between all authors. Author JAYS the study, author REDM wrote the protocol, the first draft of the manuscript, managed the literature searches, authors LCR and REDM performed the statistical analysis, authors AMG and ASH managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The purpose of this study was to determine if *Streptococcus mutans* have adapted to the polluted condition present among this population, adolescents 11-15 years of age with and without caries, and the resistance of this organism to grow at different concentrations of lead (Pb).
Methodology: In Diagram 1. Summary of the different stages of experimental development are briefly explained in the following paragraphs.
Results: The present investigation was made because contamination by chemical substances (heavy metals) represents an important health risk. We chose the community of San Baltazar Tetela, Puebla-México as our study site since it experiences noticeable environmental contamination because of industrial emissions and run-off generated by the storage of these

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polluting agents in the Manuel Avila Camacho dam (Valsequillo Lake). This study not only allowed us to quantify the concentration of lead in the blood of adolescents living in this community, but also to establish its possible association with socio-economic factors. On the other hand we isolated and identify *Streptococcus mutans* from saliva in teenagers.
The mean blood lead value has a value of 7.89 μg/dL of blood (Graph. 3) in the lower levels to 10 μg / dL, with lead levels greater 10 μg / dL of blood is 13. 18; not having statistically significant difference in applying the test, based on the weight and height of children with and without elevated lead, on the other hand the average value of CPO in children with normal blood lead was 3.99 (Graph.4), while children with higher lead levels to 10 μg / dL of blood, had a 7.41 CPO.

Keywords: Environmental pollution; lead (Pb); Manuel Avila Camacho dam (Valsequillo Lake); CPO (decay index) colony forming units (UFC).

1. INTRODUCTION

During the last twenty years there has been an increasing interest in the biological role of heavy metals in humans [1,2]. Lead is a heavy metal that is distributed widely in the atmosphere and has many uses [3,4]. Lead poisoning is common andhas been documented since ancient times [5,6]. Such poisoning has been considered an important problem in public health since it can cause kidney mutations, cerebral damage, and sudden death for those affected [7,8]. For many centuries there has been no effective treatment [9,10].

Due to the industrial development in over the last couple of centuries and accelerated urbanization, lead poisoning has become chronic [11,12]. Among its more important clinical consequences are damages related to learning, attention, and growth; the neurological damages are, for many reasons, of greater medical importance [13,14,15].

During the last decade it has been very important to monitor or detect toxic substances and drugs in saliva [16,13]. There is a close association between the toxic level of medicine and drugs in plasma and saliva [17,6,18]. In some cases more monitoring activity are performed in saliva rather than plasma. Saliva has been used to do monitoring activities and to detect microorganism that cause periodontal disease and caries [19,20].

Currently, very little information exists about the detection of polluting agents by in saliva, with the exception of mercury [21,18]. Nevertheless, greater attention has been paid to saliva because of the relation between the dental health and the polluting agents present in the atmosphere [22]. This has attracted the attention of many researchers, since the problem of lead pollution is a global problem [3,23].

2. MATERIALS AND METHODS

2.1 Criteria

Adolescents (male and female) between 11-15 years of age, students" Justo Sierra" High School, San Baltazar Tetela, Municipality of Puebla [24,13,18] [(see Map 1), which received no antibiotic treatment.

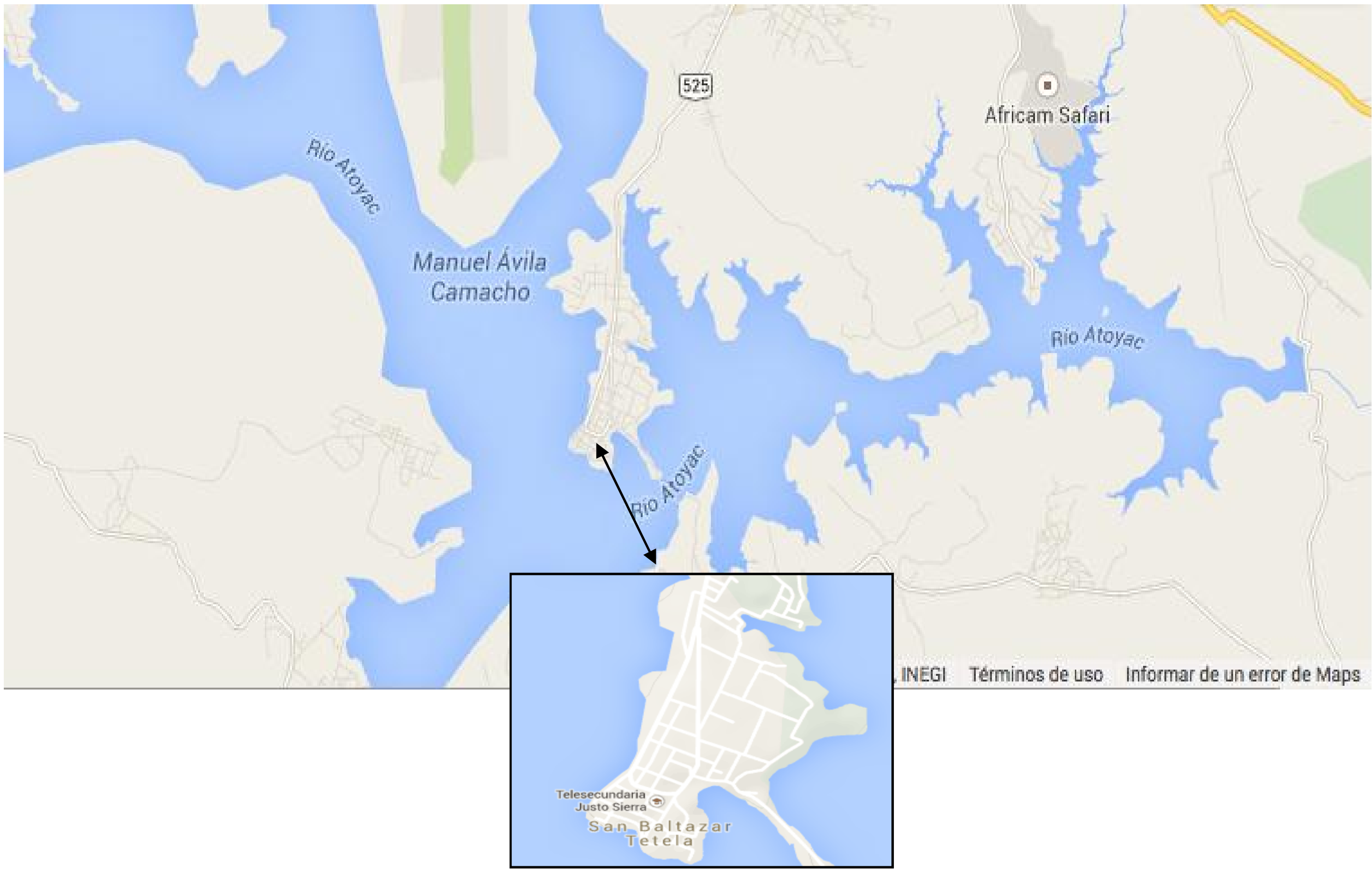
Two hundred twenty-five students" Justo Sierra" from San Baltazar Tetela High School Puebla, participated in the study. Obtaining saliva samples 210 and 158 of blood [25,20,13].

2.2 Samples

Blood samples were taken from the students to ascertain the level of lead present in these students [13,26]. Saliva samples were extremely important for isolating and identifying *S. mutans* in the junior high school population. Students filled out a clinical card detailing their dental records [16,18].

2.3 Analysis of Samples

Blood samples were taken to the Laboratory for Biological Research Institute of Science, were placed in special tubes metex change reagent, to level of lead in blood [27,14]. The same procedure was followed for each of the samples of the students a total of 158 samples were analyzed [28,12,15].



Map 1. River basin municipality of Puebla and the subbasin, M. Avila Camacho Dam / Valsequillo, Puebla, México

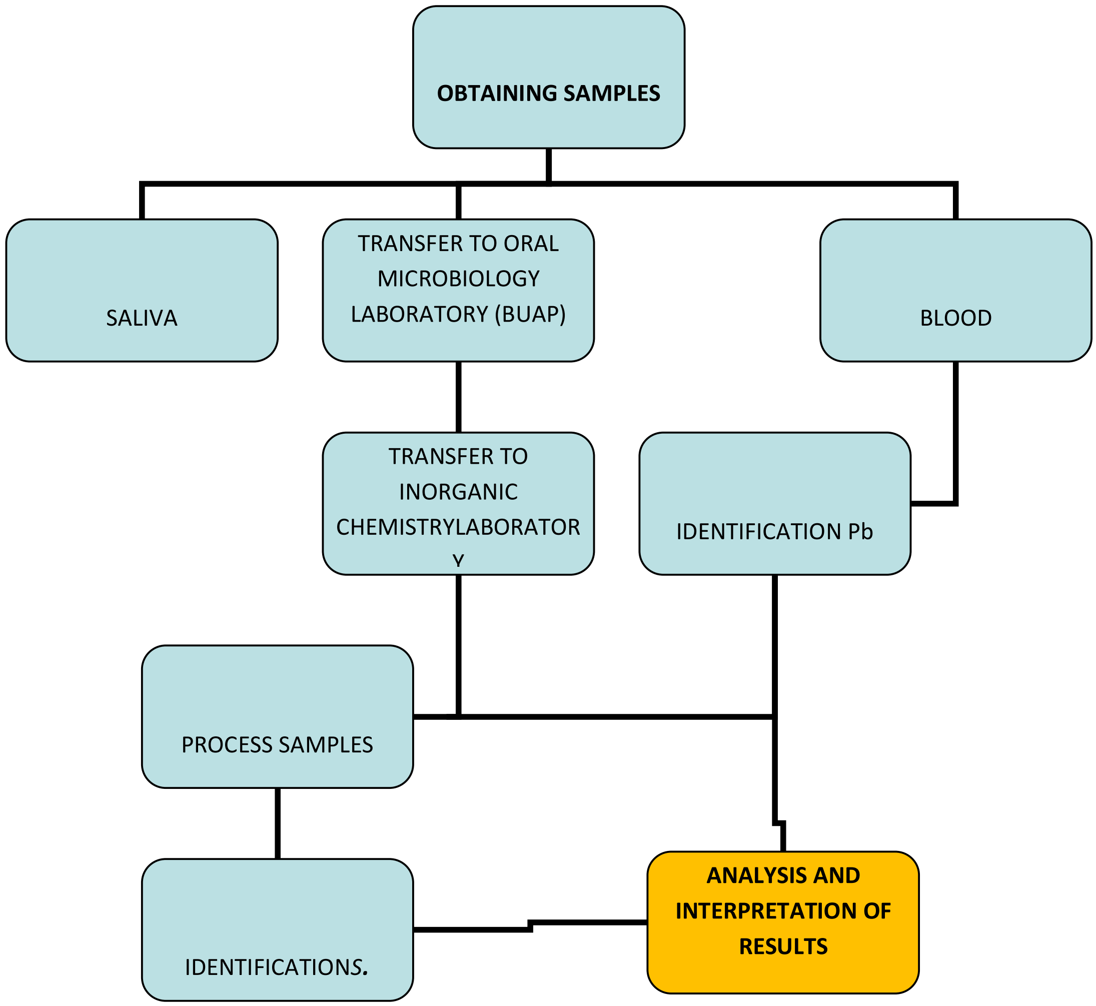


Diagram 1. Summary of the different stages of experimental development are briefly explained in the following paragraphs

Undiluted samples of saliva and two decimal dilutions, the isolation and identification of *S. mutans* was conducted using mitis- salivary us medium at the end of this procedure, which is placed in anaerobiosis for a period of 24 to 48 hours at 37°C in an atmosphere of 5% CO2 [16,17,18]. They were kept 24 hat room temperature, a period of time that is considered appropriate for the morphological identification of colonies [29,18].

2.4 Identification of *S. mutans*

Hydrolysis of arginine and fermentation of carbohydrates was done to identify *S. mutans*. For the biochemical tests, microplates with 96 wells were used for incubation for 24 hours at 37°C [30,31,32].

Once *S. mutans* was identified, each strain was cultured in Todd-Hewitt broth with sheep blood at 37°C and placed in the ultra-low freezer at -70°C [18]. The isolated strains of *S. mutans* were cultured at different lead concentrations [33,32].

2.5 Test of Resistance of *S. mutans*

The stocks of already-identified *S. mutans* were grown again in plates with the media Mitis – Salivarius to confirm the viability of the samples [19,34,35]. These were placed in jars of anaerobiosis for a period of 24 to 48hrs at 37°C in a 5% CO_2 atmosphere [34,18]. After this, they were left for another 24hrs at room temperature [16,17,18]. One colony was selected and seeded in Todd-Hewitt broth and incubated for 18 hrs. [5,18].

Lead resistance was determined by measuring the size of bacteria growth [13]. At the same time, a test was done to determine if *S. mutans* resisted different concentrations of lead acetate (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ppm). In order to determine if the strains showed metabolic activity a test was done in 96 well plates using Todd-Hewitt broth with sucrose and an indicator (phenol of red) [12]. Then 30 µl of the sample were placed in each well, immediately followed by 30 µl of lead acetate of the following concentrations0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ppm. Later, the micro plate was incubated at 37°C under 24 hrs. Then the micro plate was read, observing metabolic inhibition.

This test was done for each group of samples that was processed and then *S. mutans* was identified.

3. RESULTS

Two hundred and twenty five students were included in the study. Two hundred and ten saliva samples and 158 blood samples were processed.

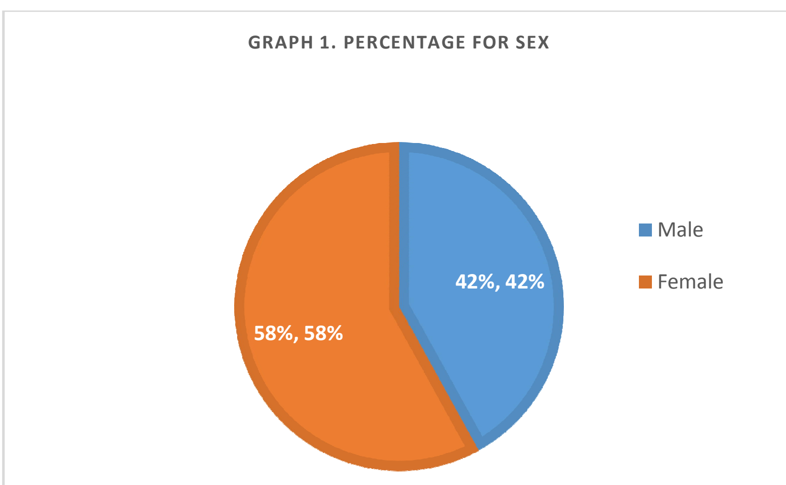
Fifty eight percent of the samples of the studied population were females, whereas 42% of the samples were males Graph 1. The percentage of lead present in the blood, corresponding to the students who presented values less than 10 µg/dL in their blood, can be noted in Graph 2.

This Graph shows that the percentage of students with a lead level less than 10 µg/dL (59.60%) is much greater than those with a lead level greater than 10 µg/dL (40.40%).

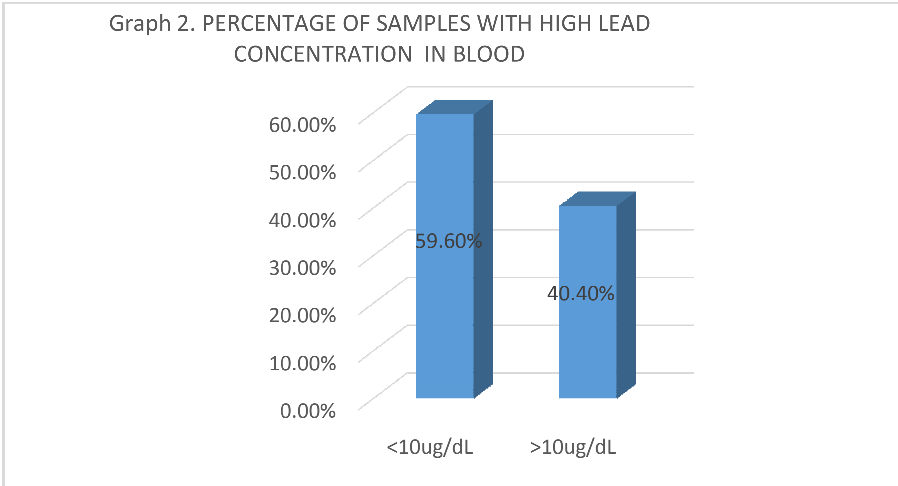
The concentration of lead in blood was 7.89 µg/dL (Graph 3.), which is smaller than the lead level of 10 µg/dL of blood; On the other hand the concentration of lead in blood with greater levels above 10 µg/dL of blood was 13.18. Nevertheless, there was not statistically difference in relation to the weight and height of the children with and without elevated lead levels. On the other hand, the value average of CPO (decay index) in children with normal values of lead in blood was of 3.99 (Graph 4.) whereas children with lead levels above 10 µg/dL of blood presented a CPO of 7.41.

Although data on the concentration of lead in the blood obtained by this study are not statistically significant, we can see that there is a relationship between the rate of caries and blood lead levels.

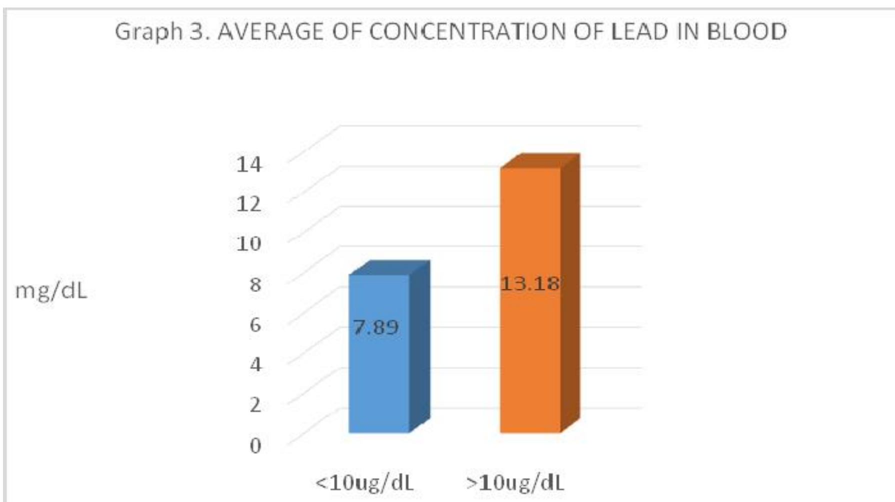
However, it does show an association of the number of colony forming units (CFU/ml) and the level of lead in the blood as an indicator of some adaptation of this organism to different levels of lead.



Graph 1. Percentage corresponding to the sex of the student population. High school "Justo, Sierra'' san Baltazar Tetela municipality of Puebla



Graph 2. Percentage of samples with high lead concentration in blood of the students of the High School "Justo Sierra", San Baltazar Tetela, Puebla



Graph 3. Calculated average blood lead samples of students of the High School "Justo Sierra" san Baltazar Tetela municipality of Puebla School

4. RESULTS OF THE SUSCEPTIBILITY OF *S. mutans* TO DIFFERENT LEAD CONCENTRATIONS

S. mutans was isolated and the number of colony forming units (CFU) was determined. The CFU of *S. mutans* from the students of first-degree group "A" and "B" is expressed in the Table 1. This result indicates the bacterial load present in the students from each group. It was possible to indicate if they were under some dental treatment or using antibiotics before collecting the saliva samples. CFU of *S. mutans* in each group presented a high concentration of lead. Owing to the variety in the oral hygiene of each student, we accounted for the nutritional habits that each student displayed (Graph 5).

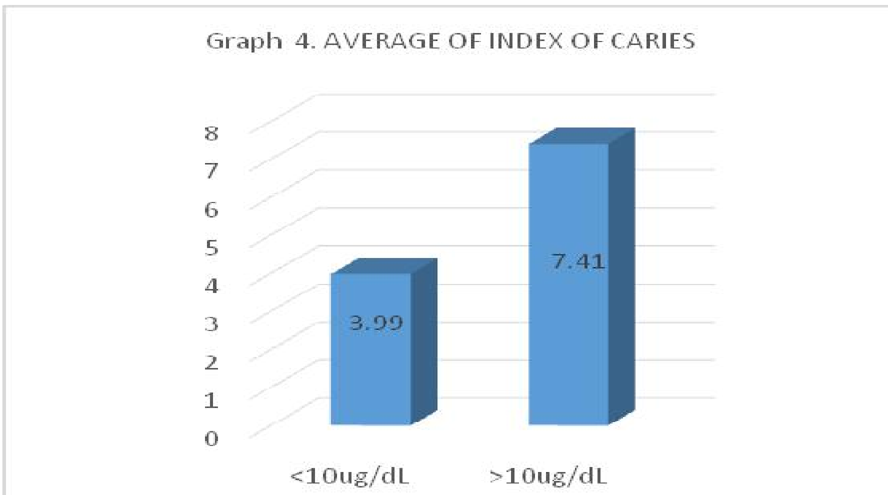
S. mutans strains showed resistance to Pb levels10 µg/gr minors. Because this was considered as the baseline value and the increased resistance of Pb was calculated according to the baseline. The "A" group obtaineda value of 100% in samples of students with higher levels of Pb 10 µg/dL blood, while the "B" group, the percentage was 19.68 (Graph 6).

The CFU of *S. mutans in* saliva is shown in Table 2 (Annex 1). The values of second grade "A", were relatively high compared to the "B" group, this group showed a significant increase (Graph 6). However, the "C" group presented very close to the "B" group values.

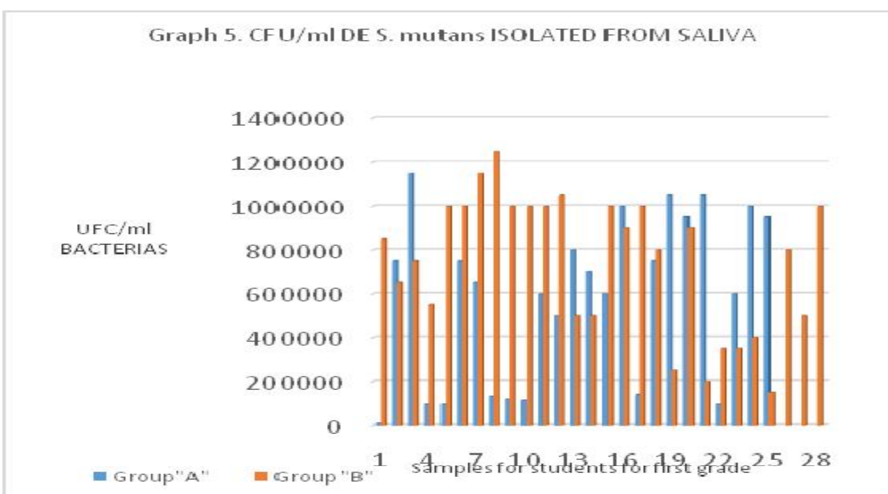
S. mutans strain showed resistance to lead levels less 10 µg/dL and expressed in CFU /ml at baseline (0%) increased the resistance of Pb was calcúlated with baseline Graph 7. Strains isolated group "A" I present resistance up to187.47% and group "B" value of155.35%.

Clearly, the values found at group "A" are much higher than those groups "B" and "C". However, the number of colony forming units (CFU/ml) for group "C" is very close to group "A". In the third grade students, the numbers of CFU/ml of *S. mutans* for both groups are shown in Table 3 (Annexed 1). These values are higher for group "A" (Graph 8).

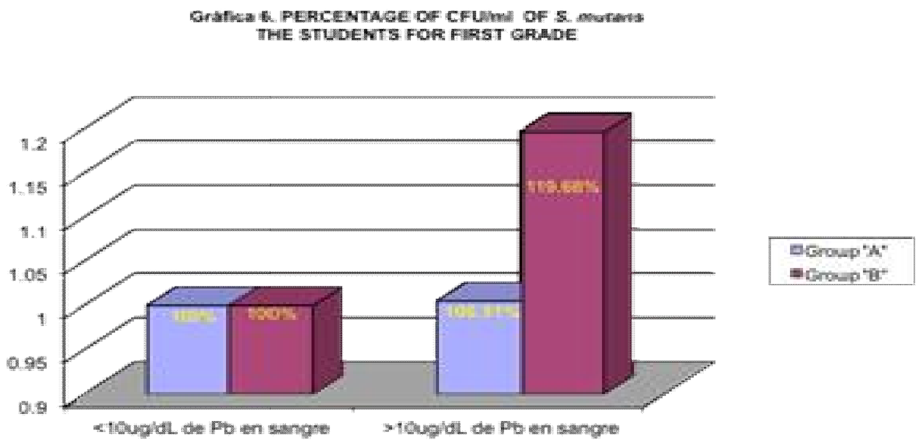
Students of the third year, the values obtained from CFU/ml of *S. mutans*, both groups are presented in Table 9 (Annex 4) these values are shown to be higher for the group "A" (Graph 9).



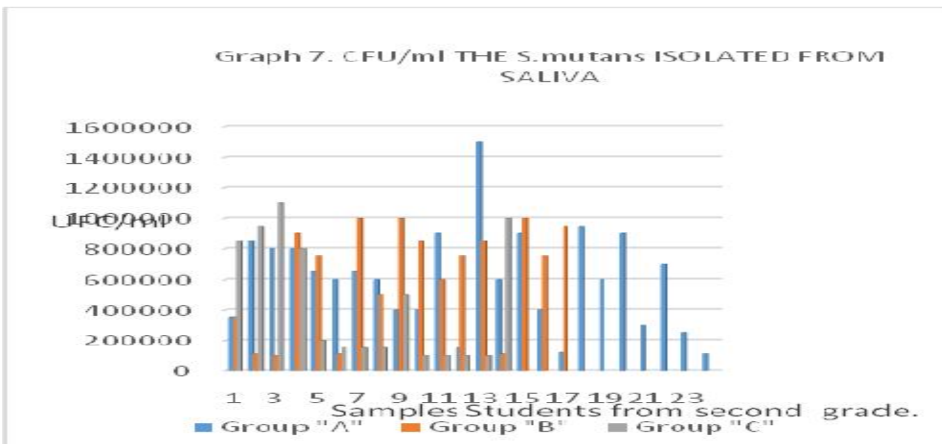
Graph 4. Average value of decay rate of samples of students. School of the High School "Justo Sierra" san Baltazar Tetela municipality of Puebla



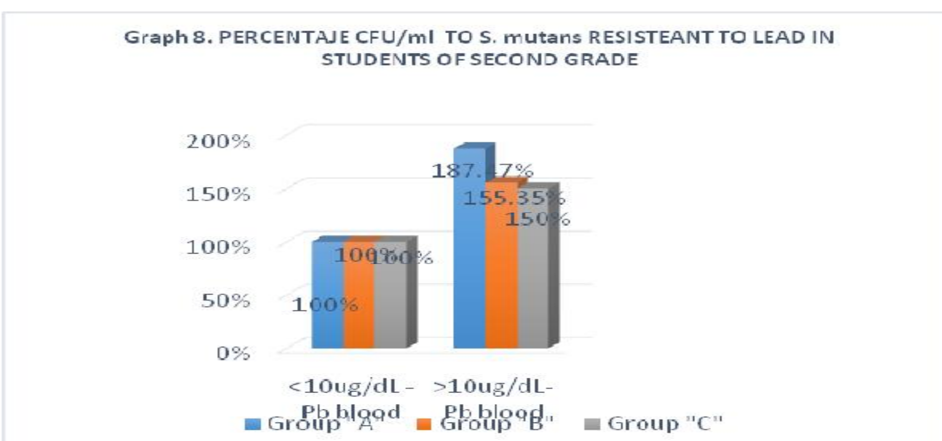
Graph 5. CFU of *Streptococcus mutans* isolated from students of the first grade group "A and B" High school "Justo Sierra" san Baltazar Tetela municipality of Puebla



Graph 6. Percentage of CFU of *Streptococcus mutans* isolated from first-year students of group "A and B". High School san Baltazar Tetela, Puebla



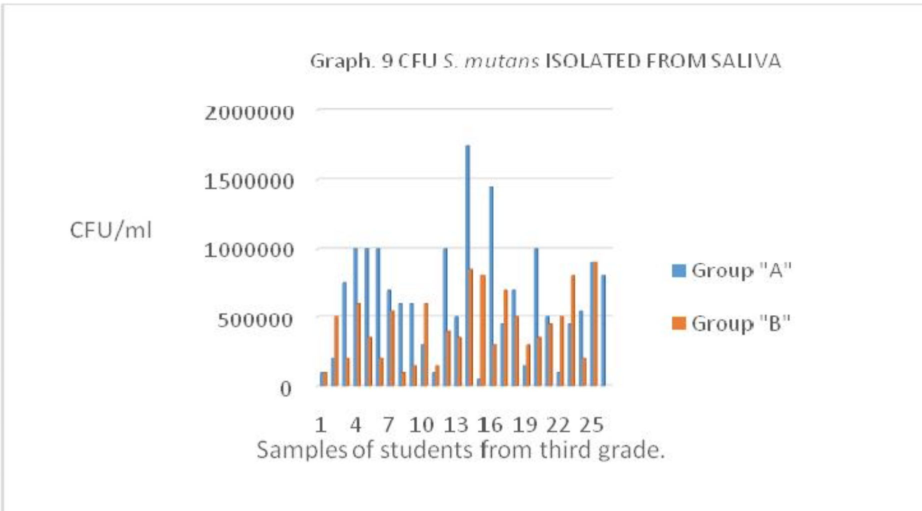
Graph 7. CFU/ml of *Streptococcus mutans* isolated from second-year students of group "A, B and C". High school Justo Sierra" San Baltazar Tetela, Puebla



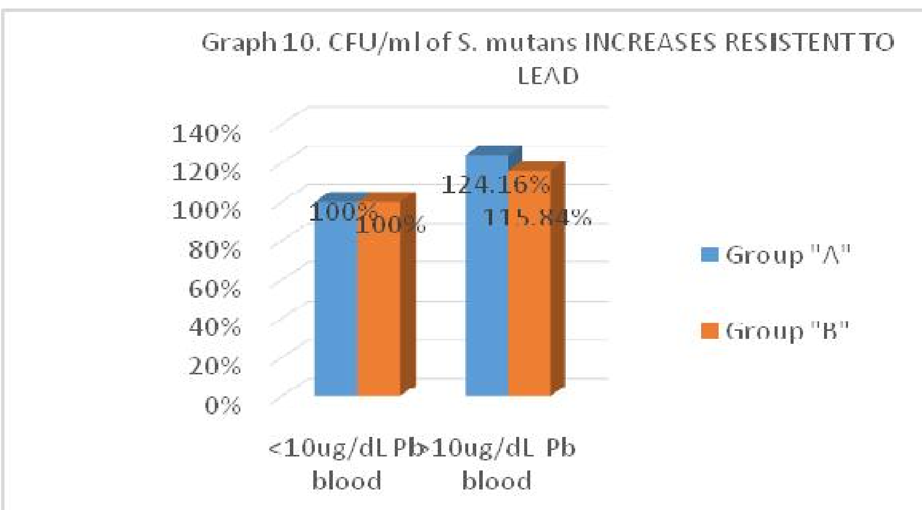
Graph 8. Percentage of UFC/ml de *Streptococcus mutans* isolated from students of the second grade group "A, B and C". High school "Justo Sierra" san Baltazar Tetela, Puebla

S. mutans strain showed resistance to 10 µg/gr lower concentrations, the increase in resistance of Pb was calculated according to the (Graph10) baseline CFU. Strains of group "A" showed resistance with a value of 124.16% and the "B" group of 115.84%.

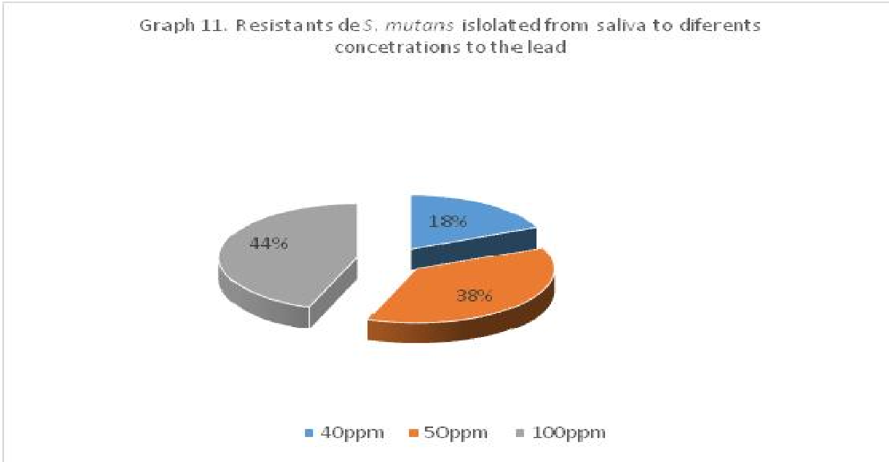
Using anti-biogram technique showed that their solated *S. mutans* strains were resistant to lead acetate concentrations of 40, 50 and 100 ppm (Graph 11). Noting a rate of 45% at a concentration of 100 ppm, taking note of the zones of inhibition at this concentration. The 50 ppm value of 37% was obtained at 40 ppm the percent age was lower than in the first two (18%) at this concentration of the isolates have a high capacity to grow. Moreover, it was observed that the samples of this group with his susceptibility to lead acetate also had a serious injury of the gums caused by the dental hygiene during the sampling and analysis of the oral health status of students (Graph 4, p. 14).



Graph 9. CFU/ml of *Streptococcus mutans* isolated from third grade students grade "A and B". High school "Justo Sierra" San Baltazar Tetela, Puebla



Graph 10. Percentage of *Streptococcus mutans* isolated from students of the third year group "A and B". High school "Justo Sierra" San Baltazar Tetela, Puebla



Graph 11. Susceptibility of *Streptococcus mutans* isolated from students of the High School "Justo Sierra" San Baltazar Tetela, Puebla

5. RESULTS ON THE LEAD LEVELS IN BLOOD AND THE INCIDENCE OF DENTAL DECAY

Table 4 (Annex 1), summarizes the data obtained from the levels of lead (Pb) and the rate of dental decay in the first grade.

Table 4 shows the index of student dental decay. These values show the incidence of dental decay that each student presented at the moment of their check-up. We can see that the number of damaged teeth goes from 4 to 12 for the group "A", whereas the number for group "B" ranges from 0 to 9.

In Table 5 (Annexed 1), the collected data of the lead levels (Pb) in blood and the index of dental decay in second-year students are shown. As we can observe, the maximum permissible level of lead in the blood of a boy, according to the Mexican Official Norm NOM-199-SSA1-2000, is <10 µg/dL(Annexed 2).Nevertheless, this level is merely safe, neither normal nor desirable, since this is the threshold above which the effects of lead appear.

The data we obtained from blood samples of sophomores are in Class I, yet the values presented in Table5 are very close to the next category (10> 24 mg/dL), which refers only to the necessary observations to follow the patient.

Table 5 shows the index of dental decay in the students during their check-ups. Here, we can see that the number of injured teeth goes from 1 to 14 in-group "A", 0 to 10 in -group "B" and 1 to 9 in- group "C". In Table 6 (Annexed 4) the levels of lead (Pb) in the blood and the index of student dental decay of the third degree appear.

Table 6 shows the index of dental decay in students during their check-ups. Here we can see that the number of injured teeth goes from 0 to 9 in group "A" and 0 to 7 in group "B".

In the following Tables we can see lead levels that are considered high according to NOM. Table 7 (Annex 1) shows high lead levels and high rates of decay of first graders. In this Table the levels of lead are located in Class II of the Official Mexican Standard (Annex 2). However, this category indicates the comprehensive medical assessments to reduce NPS (blood lead levels) and provides information to families about personal hygiene and the prevention of lead exposure, as well as information on the patient's nutrition and health reporting.

Table 7, shows the index values of student dental decay, provided by the collection of saliva and blood samples. Values indicate the oral health of each student throughout this study. The index of decay (CPO) shows the damages to the permanent dental pieces. In first-grade students, the numbers of damaged teeth were between 0 and 14, with a lead concentration of 10 µg/dL to 18.8 µg/dL in the blood, there by located in category II. However, we observed that in student group "A" values ranged from 10 µg/dL to 11 µg/dL in the blood. For the student group "B" values ranged from10 µg/dL to 18.8 µg/dL in the blood, higher than group "A".

Similarly, Table 8 (Annex 1) shows the high lead level for second graders. Here the subjects also have values ranging from 10 µg/dL to 20.3 µg/dL blood with average blood 128.3 µg/dL for the three groups, which places them in category II. But we did note that students group "A" have values ranging from 13.8 µg/dL 10 µg/dL blood. Although the students group "B" have values ranging from 10 µg/dL to 22 µg/dL blood, these values are much higher than those with the students group "A". On the other hand, the values group were obtained from "C" to 20.3 µg/dL 10.2 µg/dL ranging from blood, i.e. the values of the second graders group "B" and "C" had much higher values than those obtained by group "A".

The Index of Caries (CPO) was used to measure the damage in permanent dental pieces. In students of second-grade values, ranged from 8 to 14 damaged pieces.

The Index of Caries (CPO) for group "A" ranges from 0 to 8 injured pieces. However, for group "B" the CPO ranges from 0 to 13 damaged permanent dental pieces. In the following tables we can observe lead levels that are considered low by the Mexican Official Norm of Emergency (Annexed 1).

Table 8[a] shows the lead levels of the students of the Second Degree. These levels ranged from 1.2 µg/dL to 9.9 µg/dL in the blood, with an average of 7.5 µg/dL, which are defined as category I by the Mexican Official Norm of Emergency. However, we observed values that go from 2 µg/dL to 9.8 µg/dL in the blood of the students in the group "A". The values for the students of group "B" ranged from 6.1µg/dL to 7.3 µg/dL in the blood, higher values than those of group "A". The values obtained from group "C" range from 1.4 µg/dL to 9.8 µg/dL in the blood. Also this table shows the values of Index of Caries (CPO). For group "A," the damage observed in the permanent dental pieces of the students' ranges from 2 to 5 injured pieces. For group "B," values range from 5 to 10 damaged permanent dental pieces. Lastly, for group "C" observed values range from 1 to 7 damaged permanent dental pieces. The number of students of the second grade with higher lead levels presented values greater than those of students of the first grade.

Table 9[a], shows the low lead levels for students of the third grade, the values do not exceed the norm. The presented values range from 2.5 µg/dL to 9.8 µg/dL in the blood. Therefore these values are located in category I, with an average of 8µg/dL of blood as established by the Mexican Official Norm of Emergency. However, we observed values for the students of group "A" that ranged from 2.5 µg/dL to 9.4 µg/dL in the blood. For the students of group "B" values range from 4 µg/dL to 9.8 µg/dL in the blood, which is higher than group "A", but not far from the values of group "B". Also this table shows the values of Index of Caries (CPO). For group "A" the damage observed in the permanent dental pieces of the students ranges from 1 to 6 injured pieces. For group "B" the values on the CPO range from 0 to 14 damaged permanent dental pieces.

6. DISCUSSION

Numerous studies have identified adverse effects of lead in the nervous system, as well as the effects of high doses on the hematopoietic system, and reproductive impairment [36,11,5,37].

Other studies demonstrate that relatively low levels of lead in the blood can produce neuro-physiological upheavals in the young that affect learning ability, behaviour, intelligence and motor skills [11,23,16,14].

The problem of lead pollution affects every country in Latin America and the Caribbean (LAC), México and Peru are important producers of this metal [15,26]. The main means of lead absorption are skin and respiratory, digestive tracts and cutaneous. Absorption through the digestive tract is most important for children and adolescents [1,15,26]. Minors often explore their surroundings with the hands and mouth, and they may well ingest dirt and dust particles contaminated with lead [5,16]. Moreover, a child is able to absorb lead more easily than adults, and once inside the organisms this metal is distributed in the blood and soft tissue, stored and subsequently redistributed in bone [26]. There are no studies in México that relate the presence of lead and the ability of *S. mutans* to grow. Because of this, it was not possible to establish a discussion with previous studies. The results of this study show us that the students of this Institution had high concentrations of lead in their blood, most likely because the zone is near Valsequillo. One of the most important aspects was the different percentages between genders – 42% for males and a 58% for females. We observed that 59.60% of the students levels less than 10 µg/dL in the blood, a relatively high result, whereas the percentage of children with a lead level greater than 10 µg/dL in the blood was 40.40%. The average level of lead in the blood found in our study of the samples that presented a lead level smaller than 10 µg/dL in the blood was 7.89, whereas for the lead levels greater than 10 µg/dL in the blood the average value was 13.18.

There was no statistical difference between weight and the presence of caries in children with low or high levels of lead. We tried to associate the caries index with the lead level. Children with lead levels below 10 µg/dL showed a caries index of 3.99, whereas children with a lead level higher than 10 µg/dL showed caries index of 7.41.

Children with a high concentration of lead showed a high CPO (caries index) and children with high lead levels also had a high caries rate. It is noteworthy that there was no statistically significant difference in the weight and height of children with and without lead.

Another issue is that children who also displayed a high lead level in their blood presented a high incidence of decay, as our results show. The number of training units of colony forming unit (CFU/ml) of *S. mutans* was considerably higher than in children who had a lead level less than 10 µg/dL in their blood. This does not mean that these children did not present a high bacterial population. Perhaps diverse environmental factors played an important role in the adaptation of this microorganism. However, in the different groups studied the average levels of *Streptococcus mutans*, expressed in CFU/ml of saliva, was always greater in children with levels of lead greater than 10 µg/dL in their blood. This is significant because it correlates with that seen in the clinical (oral health). This group not only hada higher percentage of oral bacteria also problem as health and gingititis (Graph 4.) with significant bleeding gums.

Samples of children with high levels of Pb in the blood, its average value was almost double. However, in a few cases, it was found that despite a high level of lead in the blood, the rate of decay was zero and likewise the number of colony forming units was high. This suggests that the time of exposure to lead was probably longer and prevents adhesion of *Streptococcus mutans*. We also observed during this study that the strains were resistant to grow at high concentrations (40, 50 and 100 ppm) were found to be predispositions of children with high blood lead level. The number of colony forming units also was high. Therefore, future study can prove the presence of other metals, such as chromium and mercury in the blood and it could help us to understand how microorganisms can adapt to grow under different concentrations of these metals.

In our study, we noted socioeconomic deterioration of the inhabitants, and low population and economic growth. This is most likely because some of the main pollution stems is lack of proper treatment of clean water and wells.

7. CONCLUSION

Currently, lead poisoning in children is one of the major paediatric and environmental health problems in the world, which is a challenge for public health officials, clinicians, regulatory agencies and society.

Another less important measurement of prevention of lead poisoning consists of modifying food consumption to incorporate more nutrients that combat the absorption and metabolism of lead. Several studies have demonstrated that certain nutrients, including minerals like calcium, phosphorus iron and zinc, and vitamins like vitamin C, and Thiamine, can reduce the lead absorption in children [26].

Another issue that remains clear is that for the population of San Baltazar Tetela high concentrations of lead in the blood is most likely due to the town's proximity to the Valsequillo Lake In this town the average blood lead levels lower than 10 µg/dL was 7.89, while in samples with greater than 10 µg/dL concentrations, of lead in the blood was 13.18.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ANNEX

ANNEX1Results tables

Table 1. Number of colony forming units (CFU / ml) of *S. mutans,* first grade students

Sign (group "A")	UFC/ml	Sign (group "B")	UFC/ml
3	10000	1	850000
4	750000	2	650000
5	1150000	3	750000
8	100000	4	550000
11	100000	5	1000000
13	750000	6	1000000
14	650000	7	1150000
15	135000	8	1250000
17	120000	9	1000000
19	115000	10	1000000
22	600000	11	1000000
23	500000	12	1050000
24	800000	13	500000
25	700000	14	500000
26	600000	15	1000000
27	1000000	18	900000
29	140000	19	1000000
30	750000	21	800000
31	1050000	23	250000
33	950000	24	900000
35	1050000	26	200000
36	100000	27	350000
37	600000	28	350000
38	1000000	29	1000000
32	950000	34	400000
		35	150000
		36	800000
		37	500000

Table 2. Number of colony forming units (CFU / ml) of *S. mutans*, second grade students

Sign (group "A")	UFC/ml	Sign (group "B")	UFC/ml	Sign (group "C")	UFC/ml
1	350000	1	350000	2	850000
2	850000	2	1100000	9	950000
3	800000	3	1000000	10	1100000
4	800000	6	900000	12	800000
5	650000	7	750000	13	200000
6	600000	8	1100000	14	150000
7	650000	9	1000000	15	150000
8	600000	10	500000	18	150000
10	400000	13	1000000	19	500000
11	400000	15	850000	20	1000000
12	900000	17	600000	21	1000000
13	1500000	18	750000	23	1000000
14	1500000	19	850000	28	100000
15	600000	21	1100000	29	1000000
16	900000	22	950000		

Sign (group "A")	UFC/ml	Sign (group “B”)	UFC/ml	Sign (group “C”)	UFC/ml
17	400000	25	1000000		
18	1200000	26	750000		
19	950000				
20	600000				
21	900000				
22	300000				
23	700000				
24	250000				
25	1100000				

Table 3. Number of colony forming units (CFU / ml) of *S. mutans*, third grade students

Sign (group "A")	UFC/ml	Sign (group “B”)	UFC/ml
1	100000	1	1000000
2	200000	2	500000
4	750000	3	200000
5	1000000	5	600000
6	1000000	6	350000
7	1000000	7	200000
8	700000	8	550000
9	600000	10	100000
10	600000	11	150000
11	300000	12	600000
12	1000000	13	150000
15	1000000	14	400000
16	500000	15	350000
18	1750000	16	850000
19	45000	18	800000
21	1450000	19	300000
22	450000	21	700000
23	800000	25	500000
24	700000	26	300000
26	1500000	27	350000
27	1000000	28	450000
29	500000	29	500000
30	1000000	32	800000
31	450000	33	200000
32	550000	34	900000
34	900000		

Table 4. Blood lead level and caries index of first grade students

First grade group “A”		First grade group “B”	
Lead level μg / dL blood	C P O	Lead level μg / dL blood	C P O
10.6	5	8.5	6
4.4	4	6.7	2
10.5	4	10	5
2.9	8	11.6	2
9	0	11.4	2
6.4	5	8.4	4
10	3	7.6	3
8.2	10	10	5
3.9	4	13.7	8
8.8	12	7.1	3

First grade group "A"		First grade group "B"	
Lead level μg / dL blood	**C P O**	**Lead level μg / dL blood**	**C P O**
7.6	2	9.9	4
6.8	11	4.2	6
7.7	1	10	9
8.1	5	10	3
6.6	1	10.8	0
10.5	7	10	4
5.3	1	10.1	4
5.5	10	18.8	6
10.6	0	5.3	6
6.6	0	11	5
11	0	8.4	4
4.3	4	7.5	5
5.1	5	9.8	5
10.5	6	11.3	0
10.5	5	8.6	4
		4.3	4
		9.4	4
		3.9	2

Table 5. Lead level and blood caries index for second grade students

Second grade group "A"		Second grade group "B"		Second grade group "C"	
Lead level μg /dL blood	**C P O**	**Lead level μg / dL blood**	**C P O**	**Lead level μg /dL blood**	**C P O**
10.5	6	6.1	3	20.3	6
10.6	4	4.4	1	14.6	7
10	5	2.8	3	9.8	6
11	2	14.2	2	9.8	7
8.9	5	6.4	9	1.4	7
10.8	4	9.9	0	14.5	3
7.8	4	12.3	1	11.4	8
7.3	3	22	1	13.7	9
10	1	15.2	10	10.2	3
10.5	2	13.1	5	8.2	5
12	14	7.4	8	9.7	4
11.2	9	4.2	3	9.5	6
8.7	6	12	4	7.5	5
11	6	7.3	10	13.7	1
10.8	5	17.2	7		
9.8	2	10.3	5		
10.5	6	6.1	3		
8.9	5				
8.8	5				
11	2				
13.8	2				
10.1	5				
10	7				
10	5				

Table 6. Lead level and blood caries index for third grade students

Third grade group "A"		Third grade group "B"	
Lead level μg / dL blood	**C P O**	**Lead level μg / dL blood**	**C P O**
2.5	6	9.8	2
6	5	9.6	2
8.9	4	11.3	4
15.2	5	21.4	0
17.1	6	7.8	11
14.5	1	10.7	0
8.8	1	10.3	0
14.6	1	20.7	7
10.4	8	6.3	2
9.4	9	11.2	4
9.4	2	9.6	2
13.4	2	13.2	3
11.9	2	13.2	5
13.7	0	11.9	1
10.2	0	4	0
10.7	1	5.9	2
6.3	5	10.7	1
6.6	2	8.5	0
9.4	3	6.6	1
10.4	5	9.8	2
17.4	6	8.9	8
7.3	2	11.9	1
13.2	6	10.4	6
5.6	1	9.6	0
11.7	8	8.4	14
8.6	3		

Table 7. Levels of lead and high caries index first grade students

Grade	Lead level μg / dL blood	CPO
First grade group"A"	10.6	5
	10.5	4
	10	3
	10.5	7
	10.6	0
	11	0
	10.5	6
	10.5	5
	11.6	14
	11.6	5
First grade group"B"	10	5
	11.6	2
	11.4	2
	10	5
	13.7	8
	10	9
	10	3
	10.8	0
	10	4
	10.1	4

Grade	Lead level μg / dL blood	CPO
	18.8	6
	11	5
	11.3	0

Table 7[a]. Low lead levels and caries index of first grade students

	Lead level μg / dL blood	CPO
First grade group "A"	4.4	4
	2.9	8
	9	0
	6.4	5
	8.2	10
	5.1	3
	3.9	4
	8.8	12
	7.6	2
	6.8	11
	7.7	1
	8.1	5
	6.6	1
	5.3	1
	5.5	10
	6.6	0
	4.3	4
	5.1	5
First grade group"B"	8.5	6
	6.7	2
	8.4	2
	7.6	4
	7.1	5
	9.9	8
	4.2	3
	5.3	0
	8.4	4
	7.5	6
	9.8	6
	3.9	5
	8.6	5
	4.3	5
	9.4	0

Table 8. Levels of lead and high caries index second Grade students

Grade	Lead level μg / dL blood	CPO
Second grade group "A"	10.5	6
	10.6	4
	10	5
	11	2
	10.8	4
	10	1
	10.5	2
	12	14
	11.2	9
	11	6
	10.8	5

Grade	Lead level μg / dL blood	CPO
	10.5	6
	11	2
	13.8	2
	10.1	5
Second grade group “B”	10	7
	10	5
	14.2	2
	12.3	1
	22	1
	12	4
	15.2	10
	13.1	5
	12	4
	17.2	7
	10.3	5
Second grade group “C”	20.3	6
	14.6	7
	14.5	3
	11.4	8
	13.7	9
	10.2	3
	13.7	1

Table 9. Levels of lead and high caries index third grade students

Grade	Lead level μg / dL blood	CPO
Third grade group “A”	15.2	5
	17.1	6
	14.5	1
	14.6	1
	10.4	8
	13.4	2
	11.9	2
	13.7	0
	10.2	0
	10.7	1
	10.4	5
	17.4	6
	13.2	6
	11.7	8
Third grade group “B”	11.3	7
	21.4	12
	10.7	8
	10.3	8
	20.7	7
	11.2	6
	13.2	9
	13.2	13
	11.9	0
	10.7	12
	11.9	5
	10.4	6

Table 9[a]. Low lead levels and caries index third grade students

Grade	Lead level μg / dL blood	CPO
Third grade group "A"	2.5	6
	6	5
	8.9	4
	8.8	1
	9.4	9
	9.4	2
	6.3	5
	6.6	2
	9.4	3
	7.3	2
	5.6	1
	8.6	3
Third grade group "B"	9.8	2
	9.6	2
	7.8	11
	6.3	2
	9.6	2
	4	0
	5.9	2
	6.6	1
	8.5	1
	9.8	2
	8.9	8
	9.6	0
	8.4	14

ANNEX

ANNEX2 Mexican official norms

Mexican official norm	Lead
NOM-026-SSA1-1993 "Environmental Health" Assess Environment air quality with respect to the lead concentration.	Sets the allowable value for the concentration of lead in Environment applicable: • ALL THEMEXICANTERRITORY • In environmental sanitation policies regarding Human Health. • To develop ongoing and systematic investigation of the risks and damage to the health of the population, origin environmental lead contamination. The concentration of lead, as an air pollutant, must not exceed the allowable valueof1.5μg/m3over a period of3monthsarithmetic average, to protect the health of the susceptible population.
NOM-008-SSA1-1993 "Environmental Health" Paints and Varnishes Determination of soluble lead and other methods.	▪ Establishes methods for the preparation of acide xtractions required and test solutions for the determination of lead, "soluble" in paints and related liquid or powder products. ▪ The purpose is assumed that the lead content of the pigment containing lead is60% (m / m).
NOM-199-SSA1-2000 "Environmental Health" Blood lead levels and actions as criteria for protecting the health of the exposed population.	▪ Sets the blood lead levels and basic prevention and control of non-occupationally exposed population. specifications: ▪ •6.1: Criterion value for the concentration of blood Children, pregnant womenexposed10 ug/dL. 6.2: Rest of the exposed population2510μg/dL.
NOM-117-SSA1-1994 Test methods for the determination of cadmium, arsenic, lead, tin, copper, iron, zinc and mercury in food, drinking water and purified water by atomic absorption spectrometry.	Sets the test methods atomic absorption spectrophotometry for the determination of cadmium, arsenic, lead, tin, copper, iron, zinc and mercury in food, drinking water and purified water.
MODIFICACION NOM-004-SSA1-1993, Salud Environmental. Limitations and health requirements for the use and marketing of lead oxide (litharge), red lead oxide (minium) and basic lead carbonate (white lead).	This Official Mexican Standard establishes the limitations and health requirements to be fulfilled by the use and marketing of domestic and imported products containing lead oxide, red lead oxide and basic lead carbonate, either as compounds without chemical transformation and / or in the process of products that contain them to prevent harmful effects to health.

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