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Elevated levels of lead exposure and impact on the anti-inflammatory ability of oral sialic acids among preschool children in e-waste areas

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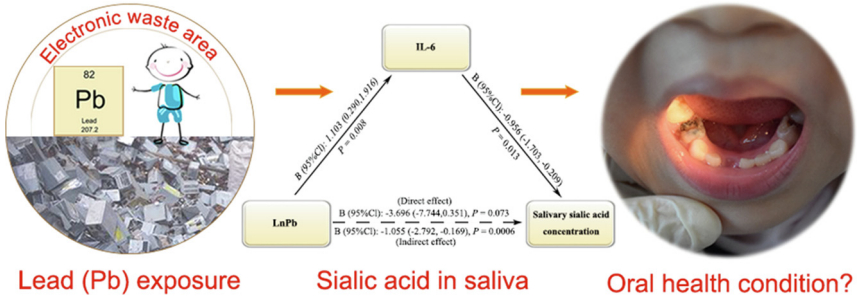
HIGHLIGHTS

- First study on the relationship of Pb and child oral anti-inflammatory ability.
- Higher blood Pb levels are associated with lower salivary sialic acid levels.
- IL-6 exerts a mediating effect between blood Pb and salivary sialic acid levels.
- Pb exposure suppresses child oral anti-inflammatory ability.

GRAPHICAL ABSTRACT



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ABSTRACT

The oral health of preschool children in an electronic waste (e-waste) area is susceptible to lead (Pb) exposure increasing the risk of dental caries and causing periodontitis and other oral diseases. The aim of the present study is to investigate the relationship between chronic exposure to Pb and oral anti-inflammatory potential of preschool children. For this analysis, 574 preschool children from 2.5 to 6 years of age were recruited between November and December 2017, in which 357 preschool children were from Guiyu (n = 357), an e-waste-contaminated town, and 217 from Haojiang Shantou. We measured the levels of child blood Pb, salivary sialic acid, serum interleukin-6 (IL-6) and serum tumor necrosis factor-α (TNF-α), and investigated the prevalence of dental caries in deciduous teeth. The medians of blood Pb levels, serum IL-6 and TNF-α were significantly higher in the Guiyu children than in Haojiang children. Concomitantly, salivary sialic acids were lower in the Guiyu children [9.58 (3.97, 18.42) mg/dL] than in Haojiang [17.57 (5.95, 24.23) mg/dL]. Additionally, the prevalence of dental caries in deciduous teeth was significantly higher in the Guiyu children than in Haojiang (62.5% vs. 53.9%). Blood Pb levels were negatively correlated with salivary sialic acids, in which IL-6 played as a mediator of the association between blood Pb levels and saliva sialic acid concentrations according to the mediation model. To our knowledge, this is the first report on the potential association between chronic Pb exposure and the anti-inflammatory ability of oral sialic acids among preschool children. These results suggest that the

Abbreviations: Pb, lead; IL, interleukin; TNF, tumor necrosis factor; E-waste, electronic waste; BMI, body mass index; B, unstandardized coefficient; CI, confidence interval; β, standardized coefficient.

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chronic Pb exposure can reduce salivary sialic acid levels, attenuate oral anti-inflammatory potential and increase the potential risk of dental caries in deciduous teeth among preschool children in an e-waste site.

1. Introduction

Despite stringent governmental regulations, lead (Pb) exposure is still of global concern. Since preschool children are in a critical stage of growth and development, Pb exposure is more likely to affect them, with blood Pb levels being elevated in and predominantly influencing young children 1–5 years of age (Carmouche et al., 2005; Chen et al., 2014; Liu et al., 2016; Lo et al., 2012; Zhao et al., 2016). Pb, a ubiquitous toxic environmental pollutant, not only impairs the respiratory and gastrointestinal systems, induces immune system disorders and increases cardiovascular disease risk, but also affects oral health (Boskabady et al., 2016; Chen et al., 2019; Farzan et al., 2018; Horton et al., 2018; Huo et al., 2019; Van de Wiele et al., 2007; Zheng et al., 2019). The oral health of preschool children plays an important role in their growth and development, formation of normal permanent dentition, normal pronunciation, and mental health. Data indicates that elevated Pb exposure is correlated with increased periodontal disease and the prevalence of caries in deciduous teeth, especially among low-income children (Arora et al., 2009; Gemmel et al., 2002; Kim et al., 2017; Wu et al., 2019). However, the specific biologic mechanism has not been identified.

Earlier studies showed that Pb exposure is associated with impaired salivary gland function. Previous papers have demonstrated that in the rat model, increased Pb exposure results in a decrease of stimulated parotid function, salivary pH and alpha-amylase enzyme activity, suggesting salivary hypofunction (Timchalk et al., 2006; Watson et al., 1997). Similarly, cigarette smoke also has a potential harmful effect on oral cavity keratinocytes by elevating intracellular oxidative stress (Aizenbud et al., 2016). Collectively, Pb could impair salivary glands to diminish salivary function. Pb exposure seriously affects the immune system by inducing oxidative stress, potentially resulting in increased susceptibility to bacteria (Dyatlov and Lawrence, 2002; Metryka et al., 2018; Sanchez-Guerra et al., 2019). Pb exposure is associated with increased levels of serum IL-6 and TNF-α and up-regulated expression of IL-6 and TNF-α mRNA (Cheng et al., 2006; Kasten-Jolly et al., 2011; Metryka et al., 2018). Thus, we hypothesize that Pb exposure may also have an effect on oral microbiota by influencing the immune system and increasing susceptibility to bacteria in oral tissue.

Some studies have observed that sialic acids play an important role in the anti-inflammatory process (Bohm et al., 2012; Spence et al., 2015). Sialic acids are a family of nine carbon backbone sugars derived from neuraminic acid that can be synthesized in the cytosol and reach high local concentrations in saliva (Lewis and Lewis, 2012; Limsuwat et al., 2016; Weidemann et al., 2010). Sialic acids are located mainly on the end of glycans of glycoprotein and glycolipid and mediate numerous cell-cell and cell-extracellular matrix interactions (Schauer, 2009). Through binding to sialic acid-binding immunoglobulin-like lectin (Siglec) on immune cells, such as siglec-7, siglec-9 and siglec-E, sialic acids participate in inhibitory signals of inflammatory responses (Bull et al., 2016; Liu et al., 2017; Spence et al., 2015). A recent study shows that the nanoparticles decorated with sialic acid produce therapeutically anti-inflammatory effects in murine and human inflammatory models (Spence et al., 2015). As it well known, long-term excessive inflammation of the oral cavity can impair oral tissue, hence the anti-inflammatory nature of oral sialic acids is critical to oral health. Sialic acids also occupy the terminal positions within salivary glycoproteins, which are of particular importance for enhancing bacterial agglutination and protecting the oral tissues from pathogens (Avsar et al., 2009; Schauer, 2009; Sprenger and Duncan, 2012).

To the best of our knowledge, there are few studies on Pb and the anti-inflammatory ability of oral cavity. Thus, we hypothesize that Pb exposure may have an impact on the anti-inflammatory ability of oral cavity by influence the immune system, and thereby increase the potential risk of dental caries in deciduous teeth.

2. Methods

2.1. Study population and data collection

For this analysis, a total of 574 preschool children from 2.5 to 6 years of age were recruited between November and December 2017, 357 participants were from Guiyu township (exposed group), an e-waste-contaminated town, and 217 were from Haojiang (reference group), a city with similar culture but with no e-waste recycling activity. Our previous research has demonstrated that Guiyu, as one of the largest Pb-exposed areas in the world, has witnessed persistent Pb exposure for over 30 years, and that Pb levels are elevated in the blood of resident children (Lu et al., 2018; Zheng et al., 2019). The two groups are similar in ethnicity, cultural background and population, but differ in exposure to electronic waste (e-waste) pollution. A questionnaire administered to each child's parents or guardians was used to record information on general demographic characteristics, child living and diet habits, residential environment, and medical histories of parent and child. Informed consent was obtained from each child's parents or guardians. This study was approved by the Human Ethics Committee of Shantou University Medical College, China.

2.2. Saliva collection and oral examination

To collect unstimulated whole saliva, participants were asked to refrain from eating or drinking for at least 1 h before saliva collection. Participants were requested to rinse their mouth with distilled drinking water to ensure that their mouth was free of food and debris before saliva collection (Goode et al., 2014; Henson and Wong, 2010; Sonesson et al., 2011). After a short rest, saliva collection was begun. All samples were collected between 8:30 and 10:30 am. Participants were instructed to sit up straight and slightly forward in their chair. A sputum cup was used to collect the saliva (Jasim et al., 2016). Each sample was stored at −80 °C until analyses. An oral health examination was conducted by trained staff on the basis of the *WHO. Oral health surveys: basic methods*. Decayed deciduous teeth were detected under natural and artificial light (WHO, 2013).

2.3. Determination of blood Pb

A total of 2 mL fasting venous blood was collected by venipuncture from subjects following a standard detailed protocol as

described previously (Zhang et al., 2016). Whole blood samples were used for measuring blood Pb concentrations. Serum samples were used for testing the concentrations of IL-6 and TNF-α. Whole blood Pb was quantified by graphite furnace atomic absorption spectrophotometry (Jena Zeenit 650, Germany). The limit of detection (LOD) was 0.051 μg/dL for blood Pb. The accuracy of the method was verified by recoveries between 91.06% and 101.2% from the spiked blood samples. Details of the blood Pb analysis have been reported in our previous studies (Guo et al., 2010).

2.4. Biomarker measurements

The concentration of salivary sialic acids was determined using a quantitative competitive ELISA kit according to the manufacturers' instructions (Biovision, USA). Detection range was 7.813–500 μg/mL, sensitivity was 4.68 μg/mL. Multiple cytokines in serum were subsequently determined with a ProcartaPlex Immunoassay Kit (Thermo Fisher Scientific, USA), all according to the manufacturers' instructions. Standard curves and sample results were generated by Luminex 200 analyzer (Luminex, USA).

2.5. Statistical analysis

Median [interquartile range (IQR)] and mean ± standard deviation (SD) were used to describe skewed distributed variables, normally distributed variables, respectively. Natural logarithmic (ln) transformation was applied to the data on blood Pb to correct the skewness. Differences between groups for normally distributed data was tested by an independent-sample *t*-test. When the data had a skewed distribution, the Mann-Whitney *U* test was performed. A chi-square test was used to compare categorical variables between different groups. A multivariable adjusted linear regression model was used to assess the association of blood Pb level with sialic acid concentrations. A mediator model was used to examine the mediation effect of IL-6 between LnPb and salivary sialic acid concentrations. Potential confounders were gender, age, body mass index (BMI), outdoor activities, the sucking/biting of toys and pencils, diet (sweet consumption, bean products, marine products), family member smoking, paternal education levels, monthly household income. Unless otherwise stated, the level of significance α was 0.05. All analyses were conducted by SPSS 22.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7.0 (GraphPad, San Diego, CA).

3. Results and discussion

3.1. Characteristics of the study population

The demographic characteristics and blood Pb level of preschool children stratified by geographic location are presented in Table 1. and Table A. 1. Children who lived in the e-waste recycling area had lower BMI and higher blood Pb levels compared with those in the non-e-waste recycling area ($P < 0.05$). Although the blood Pb level was lower than that detected in our previous research, it was significantly higher in the exposed group than in the reference group, with medians of 4.86 vs. 3.47 μg/dL, respectively ($P < 0.001$) (Xu et al., 2015). In the exposed group, >47.1% of the children exceeded the limit value of blood Pb level (5 μg/dL) set by the U. S. CDC (Betts, 2012). These results are consistent with the increase of blood lead level in children being related to poor residential environment caused by incorrect e-waste dismantling activities, as well as unhealthy living habits and the low education of parents.

Table 1
Basic characteristics of the study population.

Characteristic	Reference group (n = 217)	Exposed group (n = 357)	*P*
Age (years)	4.61 ± 0.95	4.78 ± 0.85	0.027[a]
BMI (kg/m^2)	15.42 ± 1.34	14.97 ± 1.19	<0.001[a]
Blood Pb level (μg/dL)	3.47 (2.99, 4.07)	4.86 (3.79, 6.03)	<0.001[c]
≥5 μg/dL [n (%)]	23 (10.6)	168 (47.1)	<0.001[b]

Pb, lead; BMI, body mass index. $P < 0.05$ was considered statistically significant.
[a] Mean ± standard deviation, analyzed by the independent-sample *t*-test.
[b] n (%), analyzed by the χ^2-test.
[c] Median (interquartile range), Mann-Whitney *U* test.

3.2. Effects of Pb exposure on salivary sialic acid concentrations

The median score as well as the interquartile range of salivary sialic acids is higher in the reference group than in the exposed group [17.57 (5.95, 24.23) mg/dL vs. 9.58 (3.97, 18.42) mg/dL, $P < 0.05$] (Fig. 1A). The same type of difference can be observed in the low blood Pb level group and in the high blood Pb level group, where the salivary sialic acid concentration was 13.74 (5.28, 24.24) mg/dL and 9.85 (3.97, 16.52) mg/dL, respectively (Fig. 1B).

To explore the association between ln-transformed blood Pb and salivary sialic acid levels, a multivariable adjusted linear regression model was used (Table 2). Higher LnPb was associated with lower salivary sialic acid levels in unadjusted regression analysis [B (95% CI) = −5.64 (−9.78, −1.50)] ($P < 0.01$). After adjustment for gender, age, and BMI, the negative association remained significant [B (95% CI) = −5.59 (−9.62, −1.55)] ($P < 0.01$). However, the relationship was not significant ($P > 0.05$) after adjusting for outdoor activities, sucking/biting toys and pencils, diet, family member smoking, paternal education levels, and monthly household income.

Our results show a negative correlation between blood Pb levels and salivary sialic acid levels. This is similar to results from previous studies showing that Pb and other environmental pollutants exposure can weaken salivary function and impair oral tissue (Aizenbud et al., 2016; Watson et al., 1997; Zhang et al., 2019). One possible explanation for the decreased sialic acid concentration in the exposed group is that chronic inflammation caused by Pb may influence the metabolism of sialic acids. In a previous study, decreased total sialic acid concentration was found to be related to changes in sialyltransferase activity during chronic stress which can decrease the activity of these enzymes in the liver (Gruszewska et al., 2018). Another study demonstrated that some enzymatic activities in saliva are inhibited by certain toxic components of tobacco smoke (Zappacosta et al., 2002). Similarly, Pb toxicity has been mainly linked to inflammation, immunomodulatory and oxidative stress in several epidemiologic studies (Boskabady et al., 2018; Sirivarasai et al., 2013). Also, several oral bacteria, such as some strains of *Streptoccus mitis* and *Streptococcus sanguis*, can utilize neuraminidases to release sialic acids from a diverse range of host sialoglycoconjugates and use sialic acids as nutrients (Li et al., 2012; McDonald et al., 2016). Previous studies have clearly demonstrated that chronic exposure to Pb can interfere with the balance of the gut microbiota in a mouse host (Xia et al., 2018). The oral cavity is the start of alimentary tract. Thus, we speculate that Pb exposure may also have an impact on the oral microbiota. The decreased salivary sialic acid levels associated with Pb might be a secondary effect mediated by a disorder of oral microflora. The present study indicates that the greater the Pb exposure, the lower salivary sialic acid levels, and the anti-inflammatory ability of oral cavity.

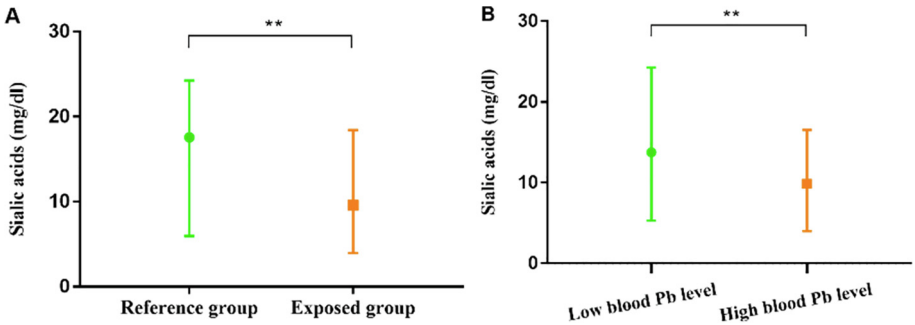

Fig. 1. A. Sialic acid concentrations in child saliva. Exposed group: n = 90. Reference group: n = 91. Results are presented as the median (interquartile range), analyzed by the Mann-Whitney U test. $^{**}P < 0.01$.B. Level of salivary sialic acids from the low blood Pb level group was higher than from the high blood Pb level group. Pb, lead. Results are presented as the median (interquartile range), analyzed by the Mann-Whitney U test. $^{**}P < 0.01$.

Table 2
A multivariable adjusted linear regression analysis for associations between blood LnPb and salivary sialic acids.

LnPb	Sialic acids	β	P
	B (95% CI)		
Model 1	−5.64 (−9.78, −1.50)	−0.20	0.008
Model 2	−5.59 (−9.62, −1.55)	−0.20	0.007
Model 3	−3.65 (−8.07, 0.77)	−0.13	0.104

Model 1: unadjusted.
Model 2: adjusted for gender, age, BMI.
Model 3: adjusted for gender, age, BMI, outdoor activities, sucking/biting toys and pencils, diet (sweetmeat consumption, bean products, marine products), family member smoking, parental education levels, monthly household income.
BMI, body mass index; LnPb, ln-transformed Pb level; B, unstandardized coefficient; CI, confidence interval; β, standardized coefficient. $^{*}P < 0.05$. $^{**}P < 0.01$.

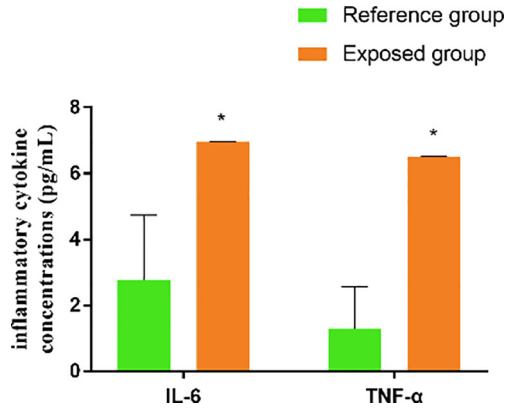

Fig. 2. Inflammatory cytokine levels in child serum. Data are presented as the median (interquartile range) and analyzed by the Mann-Whitney U test. IL, interleukin; TNF, tumor necrosis factor; $P < 0.05$ was considered statistically significant.

3.3. Pro-inflammatory cytokines and associations among blood Pb level, salivary sialic acid concentrations and pro-inflammatory cytokines

Concentrations of IL-6 and TNF-α were determined in serum samples of preschool children (Fig. 2). Compared to the reference group, children in the exposed group had a higher median level of IL-6 (6.96 pg/mL vs. 2.76 pg/mL) and TNF-α (6.51 pg/mL vs. 1.29 pg/mL) (all $P < 0.05$).

To explore whether the decrease of salivary sialic acid concentration is related to the inflammation caused by Pb exposure, a mediation analysis was used to investigated the mediation effect

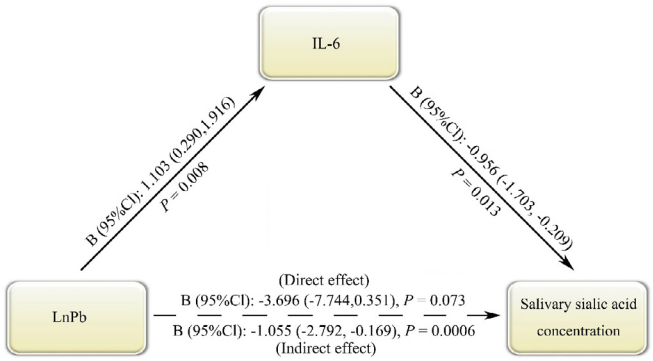

Fig. 3. Mediating effect of IL-6 on the relationship between LnPb and salivary sialic acids.LnPb, ln-transformed Pb level; IL, interleukin; B, unstandardized coefficient; CI, confidence interval.All regression analyses are controlled for age, gender and body mass index (BMI); 5000 bootstrap samples.

of IL-6 between LnPb and salivary sialic acid concentration (Fig. 3). Bias-corrected 95% CIs indicated a significant direct effect [B (95% CI) = −3.696 (−7.744,0.351)], total indirect effect [−1.055 (−2.792, −0.169)], and indirect effect through IL-6 [−0.956 (−1.703, −0.209)]. The proportion of IL-6-mediated effect in the total effect was 28.53%. No mediation effect of TNF-α between LnPb and salivary sialic acid concentration was found (data not shown). To sum up, our results demonstrate that an adverse estimated effect of Pb exposure on salivary sialic acid concentration mediated through IL-6.

It is well-established that inflammation is the main mechanism of Pb exposure-induced disorders (Boskabady et al., 2018; Farkhondeh et al., 2014; Uzu et al., 2011). Increased cytokines have also been reported in Pb-exposed humans and animal models (Boskabaddy and Farkhondeh, 2013; Boskabady et al., 2018). IL-6 and TNF-α are important pro-inflammatory factors in the inflammatory response. IL-6, mainly produced by monocytes and macrophages, together with TNF-α, involved in systemic inflammatory reactions including oral inflammatory diseases such as periodontitis (Bostrom et al., 2015; Sawada et al., 2013). Therefore, elevated levels of IL-6 and TNF-α in e-waste area children with higher blood Pb suggested that inflammatory responses are key elements involved in the reaction to environmental toxic heavy metals. The significant mediating effect of IL-6 demonstrates that the inflammatory state is conducive to Pb-associated maladaptive alterations of the oral anti-inflammatory ability. Thus, a possible mechanism for the decreased sialic acid levels following Pb exposure may be related to the inflammatory state changing sialyltransferase activity.

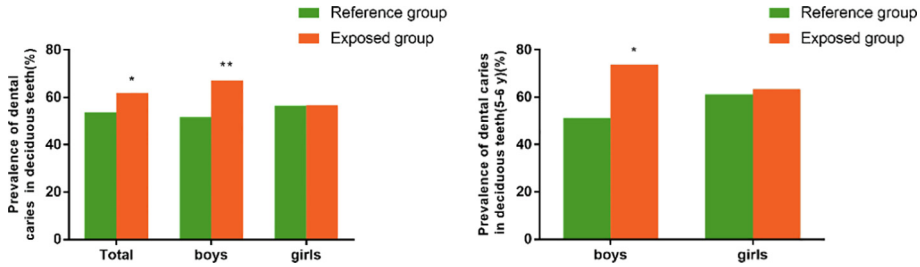

Fig. 4. Prevalence of deciduous teeth caries of 2- to 6-year-old children. Exposed group: n = 357. Reference group: n = 217. Analyzed by the χ^2-test. $^{*}P < 0.05$. $^{**}P < 0.01$.

3.4. Role of Pb exposure in impairing the oral health condition

To verify the difference in oral health between the two groups, we investigated the prevalence of deciduous teeth caries in the children from each group. The prevalence of dental caries in deciduous teeth were generally high, although it was significantly higher in the exposed than in the reference group (62.5% vs. 53.9%, respectively, $\chi^2 = 4.084$, $P < 0.05$) (Fig. 4). In the boy subgroup, the estimated prevalence of dental caries in deciduous teeth was higher in the exposed group than that in the reference group (68.0% vs. 52.4%, respectively, $\chi^2 = 7.512$, $P < 0.05$), whereas it was not statistically in the girls group. When the children were compared according to age, 5- to 6-year-old boys in the exposed group had a significantly higher prevalence of dental caries in deciduous teeth, but there were no significant differences in other age groups. It is well-established that the presence of particular microflora in the oral cavity is a critical causal factor of dental caries (Bretz et al., 2013; Struzycka, 2014). Our results indicate that the oral health condition between two groups was significant different. This is consistent with the findings of previous studies (Gemmel et al., 2002; Kim et al., 2017). However, after gender stratification, it is only significant in boys, with no association being found in girls.

Several potential limitations in this study should be recognized. Since this is a cross-sectional study, the results must be cautiously interpreted because an association, but not a causality, is shown. In addition, further research into the combined effects of environmental heavy metal toxicants and organic pollutants in larger studies of longer duration will be required to strengthen these results and confirm these associations.

4. Conclusion

In summary, this study is the first report on the potential association between chronic Pb exposure and the anti-inflammatory ability of oral sialic acids among preschool children. Our study shows that higher blood Pb is associated with a decline in salivary sialic acid levels, higher plasma levels of IL-6 and TNF-α, and increased prevalence of primary teeth caries. These results indicate that the chronic Pb exposure is a potential risk factor of the decline in oral anti-inflammatory ability among preschool children in an e-waste area.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134380.

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