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# Evaluation of lead levels in biological samples of mentally retarded children in different stages using advanced extraction method

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ABSTRACT

In present study the lead (Pb) levels has been assessed by analyzing the scalp hair and blood samples of mentally retarded/intellectual disabled (MR/ID) children of both genders, age ranged 3–8 years. For comparative purpose, healthy age matched children were also selected. The cloud point extraction of Pb from digested biological samples was carried out by complexed with ammonium pyrrolidinedithiocarbamate. The complexed analyte was subsequently isolated from the aqueous matrix in the micelles of a non-ionic surfactant (Triton X-114). Dilution of the surfactant-rich phase with acidified ethanol was performed after phase separation, and the Pb content was measured by flame atomic absorption spectrometer. Factors affecting the cloud point extraction were evaluated and optimized. The proposed procedure allowed the determination of lead in certified standard and real samples with detection limits of 0.834 $\mu g\,L^{-}$ and enhancement factor 55. The results were compared with those of healthy children have same age, socioeconomic status and residential areas.



## 1. Introduction

Lead is a neurotoxicant and has the capability of interfering with many biochemical events in cells throughout the body and can produce a wide spectrum of alterations in many organs (Mansouri and Cauli, 2009). Multiple long-term prospective and cross-sectional studies have shown that increase in blood Pb levels (BLLs) are inversely associated with learning ability, low levels of growth and delayed puberty (Mansouri and Cauli, 2009). The exposure of Pb creates adverse effects on intellectual and neuromotor performance, especially in children and adolescents (Lanphear et al., 2005). Lead interferes with signal transmission at the synapse and with

*Abbreviations:* Pb, lead; MR/ID, mentally retarded/intellectual disabled; BLL, blood Pb levels; APDC, pyrrolidinedithiocarbamate; Triton X-114, octylphenoxypolyethoxy ethanol; FAAS, flame atomic absorption spectrometry; SH, scalp hair; CRM, certified reference material; MR1, moderate mental retardation; MR2, severe mental retardation.

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cellular adhesion molecules, causing disruption in cell migration during critical times of the nervous system development (Jomes, 2009).

Young children and fetus are at particular risk for exposure to Pb, they absorb the ingested Pb from the gastrointestinal tract better than do the adults (ATSDR, 2007). Lead can cross the placenta and reach the developing brain of the fetus, whose incompletely formed blood barrier makes it more vulnerable to toxicant exposure, as compared to adults (Guilarte et al., 2003; Grandjean and Landrigan, 2007). The circulating Pb in blood access to the brain more easily in children, especially those of 5 years old or younger; and the developing nervous system in children is far more sensitive to Pb toxicity than the mature adult brains (Cory-Schlecta and Schaumburg, 2000; Grigg, 2004). Concern over the prevalence of Pb exposure has focused on the young child living in unhygienic atmosphere of industrial areas, because of their susceptibility to its adverse health effect on growth and neurobehavioral development (Lidsky and Schneider, 2003) and other physiological disorders (Chen et al., 2006; Braun et al., 2008; Shah et al., 2013).

It also appears that Pb neurotoxicity may be largely irreversible (Braga et al., 1999; Needleman, 2004). Children in less developed countries are more vulnerable to neurodevelopmental delays (because of endemic disease, caloric and micronutrient deficiencies, and limited resources for early intervention) and less likely to be examined for toxic exposures, including Pb (Durkin, 2002; Caulfield et al., 2006). However, there is accumulating evidence that Pb exposure in urban areas of developing nations is among the highest in the world (Rahbar et al., 2002; Jain and Hu, 2006; Riddell et al., 2007). The poor nutrition, low maternal education, and socioeconomic status are the factors influencing blood Pb levels in children (Mathee et al., 2002; Diouf et al., 2003), especially in low income family children (Gallicchio et al., 2002).

Whole blood is the primary biological fluid used to assess Pb exposure, both for screening and diagnostic purposes (Barbosa et al., 2005). Hair is also a biological specimen that is non-invasively collected, easily stored and transported to the laboratory for analysis (Pereira et al., 2004). It is now widely recognized that hair tissue is an accumulator for trace metals and can be a better dosimeter than blood for tracing most of the heavy metals when they penetrate the biosphere (Zaida et al., 2007).

The analysis of trace element concentrations in biological media, might be considered a difficult analytical task, due to complexity of the matrix and low concentration of these elements, which requires sensitive instrumental techniques and often a preconcentration step (Maranhao et al., 2005; Ghaedi et al., 2009). Pre-concentration can solve these problems and allows easy determination of trace elements by less sensitive, but more accessible instrumentation such as flame atomic absorption spectrometry (Surme et al., 2007; Silva and Roldan, 2009; Shah et al., 2013).

There is an emerging awareness of the importance of distribution of Pb in biological samples of children having MR/ID and healthy controls. Background and risk factor data were collected through the administration of structured questionnaires to parents of children. The preconcentration of Pb in acid digested biological samples were carried out by cloud point extraction method, complexed with ammonium pyrrolidinedithiocarbamate (APDC). Nonionic surfactant octylphenoxypolyethoxy-ethanol (Triton X-114) was used as extractant and then analyzed by flame atomic absorption spectrometry (FAAS). The different variables of CPE method were discussed briefly. The obtained resulted data are discussed in detail.

## 2. Materials and method

### 2.1. Study population

A total of 119 children (both gender) with mental retardation aged from 3 to 8 years were selected for the present study. All children were out door patients of neurological ward of civil hospital, Karachi. The IQ tests were performed on the mentally retarded subjects with the help of a psychologist, who were grouped as group II, moderate mental retardation (move normally and can learn simple communication) and group III as severe mental retardation (cannot move and talk). According to the neurologist most of the children have syndromic mental retardation and other unknown causes. Seventy normal children of same age and residential area were selected as controls. Due to restricted resources and high illiteracy rate, questionnaires were administered in local language (Urdu). The information collected included dietary habits, housing conditions, children's behavior (for example, play sites, hand-to-mouth activity, etc.) and frequency of infectious diseases, environmental and personal hygiene, parental occupations, source of consumed water, income and education. The parents of MR children told that their children have shown mental disability within the first years of life and they required full-time care by parents/attendant, which were consisted with literature reported study (Daily et al., 2000). The all understudy children were belonged to families have low socioeconomic status. Ethnically all children were Muslims. The research proposal for this study, including English and Urdu translations of the questionnaire and consent form, was submitted to and approved by the higher education commission of Pakistan. Biochemical characteristics of normal and neurological disorders subjects are given in Table 1.

### 2.2. Reagents

Ultrapure water obtained from an ELGA labwater system (Bucks, UK) was used throughout the study. Concentrated nitric acid (65%) and hydrogen peroxide (30%) were obtained from Merck (Darmstadt, Germany). Working standard solutions of Pb were prepared immediately before their use, by stepwise dilution of certified standard solution (1000 mg $L^{-1}$) Fluka Kamica (Buchs, Switzerland), with 0.2 mol $L^{-1}$ $HNO_3$. The APDC was obtained from (Fluka); and reagent was prepared by dissolving appropriate amount of APDC in 10 mL ethanol (Merck) and diluted to 100 mL with 0.01 mol $L^{-1}$ acetic acid. The nonionic surfactant Triton X-114 was obtained from Sigma (St. Louis, MO, USA) and was used without further purification. A 2% (v/v) nonionic surfactant solution was prepared by dissolving 2 mL of Triton X-114 (Merck) in 100 mL distilled water. A stock buffer solution was prepared by dissolving appropriate amounts of acetic acid and its sodium