Journal of Hazardous Materials 190 (2011) 69–74

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Betel quid chewing elevates human exposure to arsenic, cadmium and lead

Shaban W. Al-Rmalli, Richard O. Jenkins, Parvez I. Haris*

Faculty of Health and Life Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK

ARTICLE INFO

Article history:
Received 8 November 2010
Received in revised form 17 February 2011
Accepted 21 February 2011
Available online 26 February 2011

Keywords:
Areca nut
Piper betel
Arsenic
Cadmium
Lead
Bangladesh

ABSTRACT

Several studies have reported increased skin lesions in betel quid (a mixture of *Piper betel* leaves, areca nut, tobacco/flavoured tobacco, lime) chewers compared to non-chewers, exposed to arsenic (As) contaminated drinking water in Bangladesh and India. The current study has determined As, cadmium (Cd) and lead (Pb) levels of betel quids and its components using inductively coupled plasma mass spectrometry (ICP-MS). The highest concentrations of As were found in slaked lime (4.56 mg kg^{-1}) followed by *Piper betel* leaves (0.406 mg kg^{-1}) and flavoured tobacco (*zarda*) (0.285 mg kg^{-1}), with a mean concentrations of As in betel quids of 0.035 mg kg^{-1} (SD 0.02 mg kg^{-1}). Mean concentrations of Cd and Pb in ordinary quids were 0.028 (SD 0.07 mg kg^{-1}) and 0.423 (SD 1.4 mg kg^{-1}), respectively. We estimated that a daily intake of 6 betel quids could contribute 1.2, 1.9 and 8.5% of the provisional maximum tolerable daily intake (PMDTI) for As, Cd and Pb, respectively. Since betel quid chewing is most prevalent among women, our finding raises concern that women chewers – especially pregnant chewers – may be harming their health and that of their unborn babies through increased exposure to a mixture of toxic elements (As, Cd and Pb).

1. Introduction

Bangladesh and West Bengal (India) have populations that are exposed to high levels of arsenic (As) through drinking contaminated groundwater and rice. This has been linked to the development of various diseases including hyperkeratosis and skin lesions, and millions people are at risk of developing cancer [1]. The use of As rich groundwater for irrigation of crops adds to this problem, as high levels of As have been detected in rice and some vegetables grown in Bangladesh [2–4]. The As crisis in Bangladesh is well known, especially inorganic As consumption from water. The risk to human health through intake of toxic elements such as As, Cd and Pb from the food chain is also well established [5–7]. As, Cd and Pb are considered to be some of the most toxic elements in the world; they can disrupt the functioning of major human organs such as kidney and lungs. An association between Pb exposure and neurotoxicity has been reported [8]. As, Cd and Pb can increase the generation of reactive oxygen species (ROS), which may have harmful effects at the cellular and organ level [9]. Human exposure to these elements via different routes such as water and foods can lead to diverse disease processes. However, intake of these elements from non-food sources are often overlooked although they may be a contributory factor in the development of disease and this requires further investigation. In this regard a significant proportion of the Bangladeshi and Indian populations chew betel quid [10], and the possibility that this practice further increases exposure to toxic elements in communities drinking contaminated groundwater has not been previously investigated.

Betel quid chewing has been practised for hundreds of years; it is a very common social habit in East and South Asian countries. More than 200 million of the East and South Asian population chew betel quid daily [11]. The main constituents of a betel quid are *Piper betel* leaves and areca nut (the seed of the *Areca catechu* plant). It is made by wrapping chopped areca nut in a *Piper betel* leaf and some slaked lime (calcium hydroxide), whereas tobacco leaves (called *shada* in Bangladesh) or *zarda* (flavoured tobacco) are often included to improve the taste. The betel quid is commonly known as 'paan' in South Asian countries including Bangladesh and India. *Zarda* is often used in betel quids instead of untreated tobacco leaves. A relationship between betel quid chewing and increased risk of As induced skin lesions has been reported by several workers [12–14].

A significant part of the diet of the Bangladeshi community residing in the UK is composed of foods imported from Bangladesh that are sold in ethnic shops [2]. This particularly applies to fish, vegetables and betel quid components such as *Piper betel* leaves, areca nut and *zarda*. We have previously reported that As levels in Bangladeshi food is high and expressed concern that UK Bangladeshis have increased exposure to As [2]. Recently Lindberg et al. [14] determined the As content of tobacco and *zarda*, which are often included in betel quid, and highlighted that tobacco chewing can further increase the risk of As induced skin lesions among Bangladeshi people.

* Corresponding author. Tel.: +44 116 2506306.
E-mail address: pharis@dmu.ac.uk (P.I. Haris).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.02.068

The present study investigates the potential for human exposure to As, Cd and Pb through the habit of chewing betel quids. The study is particularly relevant for populations in Bangladesh and India that consume As contaminated water; some of the adverse health effects observed may be exacerbated through increased exposure arising from chewing betel quids. It is also relevant for the sizeable Bangladeshi and Indian communities residing in the United Kingdom. Furthermore, since these toxic elements can readily pass from the mother to the foetus, betel quid chewing by pregnant women may be a particular health concern. Therefore, we estimated the provisional maximum tolerable daily intake (PMTDI) for As, Cd and Pb associated with chewing betel quid.

2. Experimental

2.1. Sample collection

Different types of betel quid components that are widely consumed in Bangladesh (*Piper betel* leaves, areca nut, lime, tobacco and *zarda*), rice and vegetables were purchased from UK based ethnic shops in the cities of Leicester, Birmingham and London during the months of September 2008 and November 2009. Products analysed in this study were mainly of Bangladeshi origin and are popular among Bangladeshi communities living in the UK and in Bangladesh.

2.2. Study population

Ethical approval from De Montfort University, Faculty of Health & Life Sciences, Ethic committee, was obtained for a study investigating the dietary and life-style habits of different ethnic groups in the United Kingdom including members of the Bangladeshi community through the use of a questionnaire. From the analysis of 37 questionnaires, we found that 15 chewed betel quids. The age of volunteers was in the range of 28–71 years. All these volunteers reported to be non-smokers and did not consume alcohol. The ratio of male:female was 2.5:1 for both groups combined, but was 1:1 for the chewers.

2.3. Sample preparations

All glassware and plastic were cleaned by soaking them in 10% HNO_3 for at least 12 h and then rinsed several times with double distilled water.

2.3.1. Piper betel leaves and other food preparation

Piper betel leaves were washed three times with deionised water, dried in an oven at 80 °C for overnight, and then ground with a grinder. Other samples (areca nut, rice, tobacco and *zarda*) were also ground after drying, but without prior washing.

2.3.2. Betel quid preparation

Some betel quid samples (ordinary and sweet) were collected from ethnic shops in the UK (Leicester, London and Birmingham). However, betel quids were also prepared in the laboratory by combining different chewing components in proportions that are commonly used in commercial preparations and also based on information obtained from betel quid chewers. There is no literature data on precise quantities of the various components of betel quids that make up a typical betel quid. In our study we used one leaf (approx. 1 g dry weight), combined with areca nut (approx. 4 g), slaked lime (approx. 0.4 g) and tobacco (0.4 g—either tobacco alone or *zarda*). The quantities of these materials used in the quid can vary and some chewers do not include tobacco or slaked lime in their quids. An ordinary quid typically contains *Piper betel* leaf, areca nut, some slaked lime and some tobacco. A sweet quid contains similar components as an ordinary quid but also includes sweet flavoured components such as *pan masala*, coconut, cumin, flavoured *zarda*, etc. The betel quids were dried overnight in an oven at 80 °C and then ground with a food grinder before digestion (see Section 2.3) and subjected to ICP-MS analysis (see Section 2.4). The dry weight of betel quids ranged from 5.5 to 11 g with an average of 7 g. This average weight, together with the mean concentration of As, Cd and Pb in ordinary betel quids, was used for calculating the provisional maximum tolerable daily intake (PMTDI) for these elements. Some betel quid chewers ingest the whole betel quid and we have used this scenario for our calculation. However, some chewers do not ingest the entire betel quid and may spit out excess juice and/or solid material left over after extensive chewing.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) [15], method was used to determine the PMTDI of toxic elements from betel quids. The unit used for this scale is mg of element per day. The average adult body weight of the Bangladeshi women was taken to be 60 kg [15].

2.4. Sample digestion

Ordinary and sweet betel quids and their individual components were digested by microwave assisted digestion in ultra pure 70% HNO_3 (Romil-UpA, Ultra Purity acid). A ground weight (0.2–0.4 g) of sample was mixed with 4 mL of 70% nitric acid and 2 mL H_2O_2 and then digested for 40 min using a microwave digester at a total pressure of 20 bars and a maximum temperature of 150 °C (power was 800 W) (Anton Paar—Multiwave 3000 Microwave Sample Preparation System, Austria). The solution was evaporated and then made up to 25 mL in volumetric flasks with ultra pure water (Romil-UpS, Ultra Purity water) for analysis.

2.5. Determination of total As, Cd and Pb content

Elements in the digested sample solutions were determined by inductively coupled plasma mass spectrometry (ICP-MS) [Thermo-Fisher Scientific X-SeriesII] at the University of Nottingham (UK), equipped with CCTED (collision cell technology with energy discrimination); internal standards were scandium (50 $\mu g\,L^{-1}$), rhodium (10 $\mu g\,L^{-1}$) and iridium (5 $\mu g\,L^{-1}$) in the preferred matrix of 2% HNO_3. For calibration, external standards for each element were prepared in the range of 0–100 $\mu g\,L^{-1}$ (ppb), with sample introduced via an autosampler (Cetac ASX-520) through a concentric glass venture nebuliser (Thermo-Fisher Scientific). The data processing was undertaken using Plasmalab software (version 2.5.4; Thermo-Fisher Scientific).

2.6. Quality control and standard reference material

In this study, all the sample masses were measured to an accuracy of ±0.1 mg. Elemental concentrations obtained by ICP-MS technique were evaluated by the use of certified reference materials and were found to be in good agreement with the certified values of the references material. The analytical procedure and the reliability of the digestion process of betel quid components: excluding slaked lime, were validated by analysis of NIST standard reference material (tomato leaves NIST 1573a SRM, USA). Average recoveries of As and Cd from the reference material were 92, 89% of the certified values (certified values of As and Cd are 0.112 ± 0.004 and 1.52 ± 0.04 mg kg^{-1}), respectively. However, for Pb a certified reference material (Seaweed CRM 9, NIES, Japan) was used, and the recovery was 93% of the certified values (certified value of Pb is 1.35 ± 0.05 mg kg^{-1}). Levels of elements in slaked lime were validated by analysis of Montana I soil (2710a) standard reference material; recoveries of As, Cd and Pb were 93, 90 and 101% of the certified values (certified values of

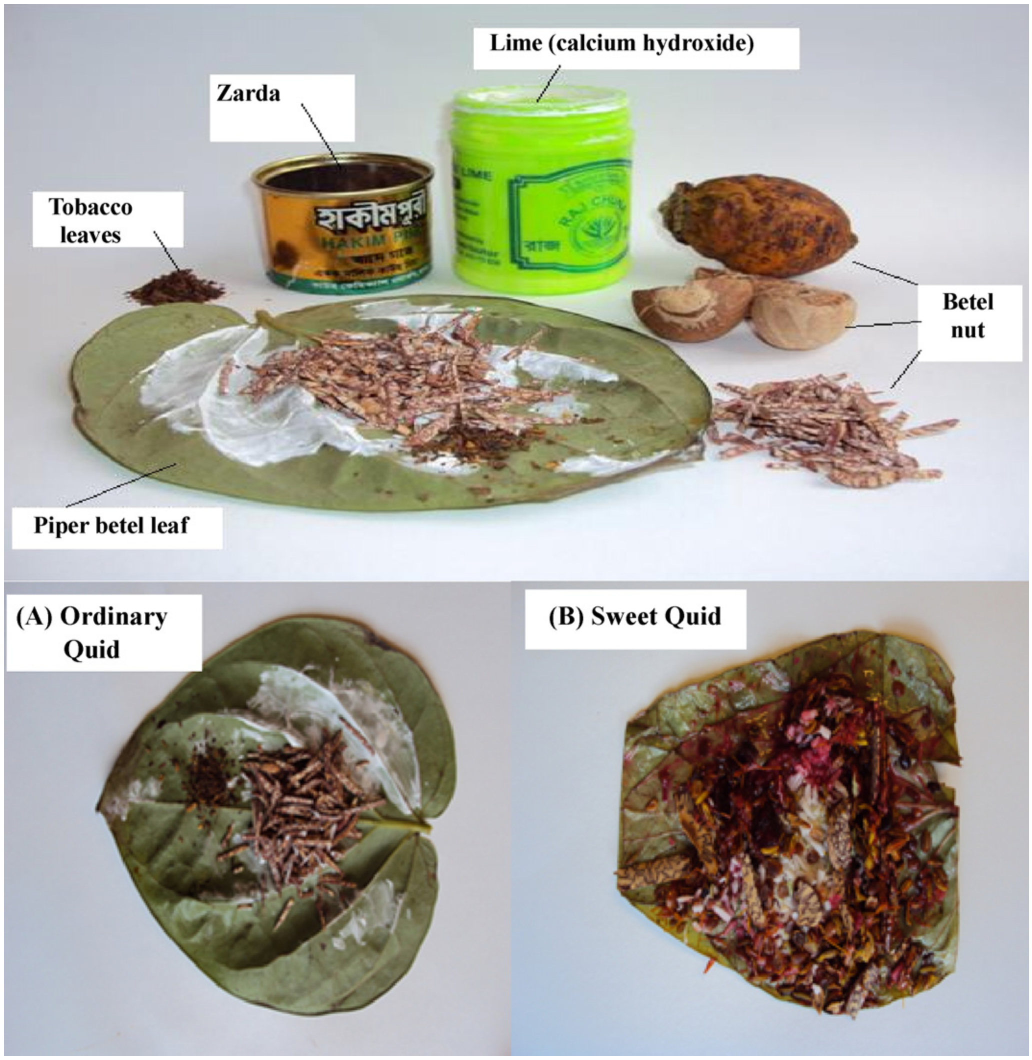

Fig. 1. The key components of a betel quid: areca nut (Betel nut), *Piper betel* leaves, slaked lime (calcium hydroxide), *zarda* (flavoured tobacco) and tobacco leaves. (A) An ordinary betel quid prepared for chewing and (B) a sweet betel quid prepared for chewing.

As, Cd and Pb are 1540 ± 100, 12.3 ± 0.3 and 5520 ± 30 mg kg^{-1}), respectively.

3. Results

Fig. 1 show photographs of typical betel quids (ordinary and sweet) and the key components of the betel quid namely areca nut, *Piper betel* leaves, slaked lime, *zarda* and tobacco leaves.

ICP-MS determined concentrations of As, Cd and Pb in betel quids and their various components are shown in Table 1. The dry weight of betel quids ranged between 5.5 and 11 g with an average of 7 g. The standard deviation (SD) was also determined and the mean concentrations of As, Cd and Pb present in betel quids were used for calculating the PMTDI for each of these elements.

The mean concentration of As in whole betel quids was higher for ordinary quids (0.035 mg kg^{-1}) compared with sweet quids (0.02 mg kg^{-1}). With regards to the individual components, the descending order based on mean levels of As were: slaked lime > tobacco leaves > *zarda* > *Piper betel* leaves > betel nut. The highest concentration of As was found in slaked lime (4.56 mg kg^{-1}). Mean levels of As in slaked lime were 23-fold higher than in tobacco leaves. The mean concentration of As in *Piper betel* leaves showed a particularly wide range of As concentrations, with the highest level in the *Khasia* variety (0.406 mg kg^{-1}). Thus the *Piper betel* leaf, which is the main characteristic component of a betel quid and considered by many to be healthy due to its "green" colour (analogous to eating green vegetables), may contribute a significant proportion of ingestible As depending on the variety used. Estimated intake of As through foods, excluding drinking water, is 74.2 μg in the Bangladeshi population, chewing 6 betel quids can contribute up to 2% of the daily intake of As (Al-Rmalli et al., manuscript under preparation).

Of the different betel quid components analysed, *zarda* had the highest levels of Cd and Pb, at 1.16 and 53.2 mg kg^{-1}, respectively. The highest concentrations of Cd and Pb in betel quids were 0.065 and 5.27 mg kg^{-1}, respectively. However, the mean concentrations of Cd and Pb in a single betel quid were found to be 0.028 and 0.423 mg kg^{-1}, respectively. Estimated intake of Cd and Pb through foods, excluding drinking water, is 34.5 μg and 75 μg in the Bangladeshi population, chewing 6 betel quids can contribute upto 3.3% and 25% of the daily intake of these elements, respectively (Al-Rmalli et al. manuscript under preparation).

Table 2 presents the estimations of PMTDIs for As, Cd and Pb through ingestion of individual ordinary betel quids. It has been reported that betel quid consumptions in a Bangladeshi population ranges from 5.7 to 6.3 per day [1]. This is higher than that reported for a Bangladeshi population living in the UK, with an average of

Table 1
Concentrations of As, Cd and Pb (mg kg^{-1} dry weight) in betel quid chewing components.

Component	*n*	Mean	Std. dev.	Median	Minimum–maximum
Ordinary betel quid	26				
As		0.035	0.02	0.033	0.011–0.082
Cd		0.028	0.01	0.028	0.007–0.065
Pb		0.423	0.99	0.204	0.100–5.270
Sweet betel quid	12				
As		0.020	0.02	0.013	0.007–0.070
Cd		0.014	0.01	0.010	0.006–0.042
Pb		0.630	0.53	0.370	0.140–1.650
Piper betel leaves	30				
As		0.107	0.08	0.092	0.013–0.406
Cd		0.049	0.04	0.030	0.006–0.155
Pb		0.725	0.46	0.630	0.258–2.259
Areca nut (betel nut)	7				
As		0.013	0.01	0.013	0.002–0.030
Cd		0.016	0.01	0.017	0.003–0.045
Pb		0.102	0.05	0.100	0.057–0.153
Lime (Calcium hydroxide)	6				
As		3.470	0.98	0.291	1.863–4.560
Cd		0.131	0.04	0.062	0.073–0.166
Pb		1.551	0.12	0.960	1.371–1.590
Tobacco leaves	2				
As		0.151	0.05	0.151	0.116–0.186
Cd		0.641	0.01	0.641	0.638–0.645
Pb		1.06	0.32	1.060	0.840–1.290
Zarda (flavoured tobacco)	13				
As		0.150	0.07	0.142	0.040–0.285
Cd		0.402	0.32	0.378	0.030–1.160
Pb		4.82 (0.78)[a]	14.6	0.830	0.220–53.50

Std. dev., standard deviation.
[a] Mean of *zarda* samples without one brand which has high Pb content (53.50 mg kg^{-1}).

2–5 quid per day [16]. In our survey of 37 Bangladeshis, we found an average of 3.5 betel quids per day with a range of 1–30 per day (manuscript submitted). Other studies have also reported that people can chew up to 30 quid per day [17,18]. In our study, we chose a more modest intake of six quids per day for the various calculations. As shown in Table 2, consumption of 6–30 betel quids per day (assuming a Bangladeshi body weight of 60 kg) contributes to 1–6% of the PMTDI, since the PMTDI of inorganic As is 2.1 μg kg bw^{-1} day^{-1} [15]. For Cd and Pb, 1.9 and 8.5% of the PMTDI, respectively, can be attributed to chewing 6 betel quids per day (Table 2). Of particular note is that chewing 30 quids per day is estimated to contribute almost half of the PMTDI for Pb.

4. Discussion

Very little research has been carried out on the intake of As and other toxic elements from non-food sources such as betel quid chewing, in populations that are exposed to As through drinking water. Betel quid and their components are widely consumed in As contaminated regions of Bangladesh and India. Exposure to As, Cd, Pb and other elements from betel quids have not been previously considered despite the fact millions of people in these regions consume them on a daily basis. Although a few studies have investigated As intake from various types of foods consumed in Bangladesh [3,4], these studies did not include contribution from betel quids. Hence, it is very important to monitor the impact of this chewing material on different populations.

Many diseases have been associated with the chewing betel quid including oral cancer (particularly cancer of the buccal and labial mucosa) [19,20] and oral submucous fibrosis [21]. Chewing *Piper betel* leaves and betel nut, either with or without tobacco, can cause cytogenetic changes in the oral epithelium; it is a strong factor for development of oral submucous fibrosis and mouth cancer [22]. Among South Asian communities in the UK, 42% of the 50–80 year age group chew *Piper betel* leaves and areca nut with and without tobacco, and they also use slaked lime [23,24]. We found that slaked lime, which is mainly composed of calcium hydroxide, has the highest concentration of As among the different betel quid components (4.56 mg kg^{-1} with a mean of 3.47 mg kg^{-1}). From our discussions with chewers we have learnt that heavy chewers can consume as much as 100 g of slaked lime per week. Furthermore, the level of As in *Piper betel* leaves is high. These leaves along with areca nut are the two key essential component of a betel quid. The intake of As from slaked lime and *Piper betel* leaves have not been previously reported. Earlier studies on the relationship between betel quid chewing and health outcomes have been mainly focused on tobacco and areca nut, with little attention been paid to assessment of the harmful effects of *Piper betel* leaves or slaked lime. It has been reported that 78% of Bangladeshi adults chew betel quid, with significantly more women than men chewers [23]. Another study

Table 2
Percentage of PMTDI for As associated with consumption of betel quids.

Element	PMTDI (μg kg bw^{-1} day^{-1})[a]	PMTDI (μg day^{-1})[a]	Percentage of PMTDI in ordinary betel quid[b]			
			3 quids	6 quids	10 quids	30 quids
As	2.1	126	0.58	1.2	1.9	5.8
Cd	1.0	60	1	1.9	3.2	9.8
Pb	3.5	210	4.2	8.5	14	42

[a] The numerical values shown are the tolerable daily intake for a 60 kg person derived from PMTDIs recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [15].
[b] Ordinary betel quid are more commonly chewed in Bangladesh, especially at home.

reported that Bangladeshi men in UK have a high prevalence of hyperhomocystinemia, compared to their white counterparts, with betel quid chewing and smoking as strong risk factors [25,26]. The fact that betel quid chewers who consume As contaminated drinking water in Bangladesh have a higher incidence of skin lesions [12] led us to investigate if betel quids contain significant levels of As, which may be a contributory factor in the development of adverse health impacts in humans. Fortunately for UK Bangladeshis, exposure to As intake from drinking water is not a problem; unlike the population in Bangladesh where an average content of up to 0.084 mg L^{-1} of As in groundwater has been reported [27].

Our findings adds further support to the study by Lindberg et al. [14] who investigated the effect of smoking and tobacco chewing on the health of a Bangladeshi population. Although they did not analyse individual components of betel quids, they determined the As content of different types of tobacco and *zarda* that are included in betel quids. They found levels of As in these products that are 50% higher compared to our values, which could be due to analysis of different varieties and batches of *zarda* and tobacco. Lindberg et al. [14] did not determine the As concentration of *Piper betel* leaf, which is the main component – along with areca nut – in betel quid preparations. In our study we have found that the highest As concentration was in *Piper betel* leaves and slaked lime, not in tobacco or *zarda*. Lindberg et al. [14] stated in their paper that chewing tobacco is associated with a greater risk factor for As related skin effects in some women and that this could be associated with poor metabolism of As in these women. In light of our findings, we believe that those women who are heavy chewers of betel quids are at risk of increased exposure to As, Cd, Pb and other substances including organic compounds. This makes them more vulnerable to toxic compound induced ill health. Possible additive or multiplicative effects of exposure to these toxic elements may provide an explanation for the higher skin related disease among betel quid chewers. ROS can be generated by these elements, which may cause toxic effects at the cellular and organ level.

Whilst exposure to As and its health impact on the Bangladeshi populations is being extensively studied very little research has focused on Cd and Pb exposure. Elevated exposure to Cd has been associated with diverse disease processes such as renal effects, bone effects and liver and kidney cancers [28]. It has been reported to mimic estrogen [29] and may increase the risk of hormone related cancer in women [30]. Vahter and co-workers [31] have been addressing the issue of Cd exposure in Bangladesh and found higher Cd levels in breast milk in Bangladeshi women. The mean concentration of Cd in breast milk in Bangladeshi women was found to be 0.14 $\mu g\,L^{-1}$, which was higher than other countries excluding Japan and India [31]. Plant based foods, especially vegetables and rice, are the main source of Cd exposure. In our view, the Bangladeshi population with a dominant plant based diet, especially the poor who cannot afford animal products and do not have a balanced and nutritious diet, are likely to be particularly vulnerable to increased Cd exposure and this situation will be further exacerbated for those who chew betel quid. Thus, it is possible, that the high consumption of betel quids by Bangladeshi women is a contributory factor for the elevated Cd exposure resulting in higher levels of this element in their breast milk, which can be harmful to their health and that of their breast-fed child. Kile et al. reported that pregnant women in rural Bangladesh are exposed to elevated levels of a mixture of elements including Cd and As and Pb that warrants further investigation [32]. Kazi et al. recently reported that betel quid chewers who use tobacco were exposed to higher levels of Cd [33].

Exposure to elevated levels of Pb has been associated with various diseases including lungs cancer [34], immunotoxicity [7] and neurotoxicity [8]. Due to increased industrialisation and use of Pb based fuels and chemicals, exposure to Pb in Bangladesh is becoming a significant problem and elevated blood Pb levels have been detected in primary school children in Dhaka (Bangladesh) [35]. A very high level of Pb (as much as 53.2 mg kg^{-1}) was detected in one brand of *zarda*. The source of Pb could be the tobacco leaves or in the chemicals that are used for treating the tobacco leaves to produce *zarda*. This explains the very high standard deviation seen in Table 1 for Pb. Kile et al. recently reported high levels of Pb exposure in women and new born babies in rural Bangladesh [32]. From our study, 8.5% of the PMTDI for Pb can be derived from chewing 6 betel quids per day. This intake from betel quids chewing may result in the PMTDI for Pb being exceeded for Bangladeshi populations if we consider that some cities of Bangladesh have high levels of Pb in their air due to traffic pollution, in addition to exposure from vegetables and rice which can contain higher levels of Pb compared to animal products. Thousands of economically deprived Bangladeshis reside in slums of the capital city Dhaka, often adjacent to roads, and are exposed to toxic elements including lead from traffic pollution. This, along with the absence of a balanced and nutritious diet, are factors likely to make individuals more vulnerable to exposure to Pb, especially those who also practice the habit of chewing betel quids.

Considering that betel quids contain significant levels of As, Cd and Pb, the possible health impacts associated with increased exposure to these elements in regular consumers of betel quids needs to be taken into account in any public health improvement strategies. Millions of people in Bangladesh and India have a diet that is dominated by plant based products, especially vegetables and rice, and their consumption of animal products is either very limited due to economic reasons or is entirely absent due to religious and/or cultural reasons. This makes them more vulnerable to higher exposure to As, Cd and Pb as these elements are present in higher levels in plant based foods. The situation that can be further worsened by the habit of chewing betel quids, which can contribute significantly towards exceeding the total permitted daily intake of As, Cd and Pb. It is possible that betel quid chewing is one of the factors responsible for the observation that women in rural Bangladesh are exposed to elevated levels of Mn, Cd, Pb and As [32]. Exposure to high levels of a mixture of these elements may result in various diseases including adverse neurological effects, kidney disease, oral cancer and submucous fibrosis etc. Although the mechanism underlying the disease needs to be investigated, it is possible that the higher incidence of skin lesions and other adverse health outcomes in betel chewers in As exposed regions may arise due to adverse additive or multiplicative effects, such as oxidative stress, induced by exposure to a cocktail of chemicals including As, Cd and Pb. The additional daily burden of exposure to As, Pb and Cd from betel quids may exacerbate the situation for chewers. Although our study has focused on toxic elements such as As, Cd and Pb, it is possible that other inorganic and organic components play a role and this aspect needs to be investigated in the future. Further studies are necessary to determine the bioaccessibility and bioavailability of As, Cd and Pb from betel quids in order to obtain a more accurate risk assessment. Studies in our laboratory are in progress to determine which types of As species are present in betel quids, and its individual components, so that a better estimation of risk to human health can be evaluated.

5. Conclusion

This is the first study to evaluate the total content of As in betel quids, analysing each of its major components, so that its contribution to the daily intake of arsenic can be established. Slaked lime and *Piper betel* leaves were identified as containing the highest As levels. Betel quids can provide between 1 and 6% of the total daily intake of arsenic for someone chewing between 6 and 30 quids per day. In addition to As, we found significant levels of Cd and Pb in different components of betel quids. The additive or multiplicative adverse effects, such as generation of ROS, may result from the simultaneous exposure to As and Cd, making chewers more vulnerable to ill health. Whilst we cannot be certain that the intake of As, Cd and Pb from betel quids can explain the increased incidence of skin lesions and other adverse health outcomes in chewers, the increased exposure may be sufficient to tip the balance towards development of disease processes. It is possible that As, Cd and Pb in synergy with organic substances in betel quids may be responsible. Those with a mainly plant based diet, that often contain higher levels of As, Cd and Pb compared to animal products, are at greatest risk if they further elevate their exposure to these elements through betel quid chewing. Betel quid chewing is most prevalent among women who may be unknowingly harming their health and that of their unborn babies (for those who are pregnant) through increased exposure to As, Cd and Pb.

We consider that betel quid consumption, especially those consuming large number of quids per day, are at risk of developing ill health and public health policy makers should seriously consider implementing policies to reduce or eliminate the consumption of betel quids in populations where this practice is prevalent.

References

[1] D. Hafeman, H. Ahsan, T. Islam, E. Louis, Betel quid: its tremor-producing effects in residents of Araihazar, Bangladesh, Mov. Disord. 21 (2006) 567–571.
[2] S.W. Al Rmalli, P.I. Haris, C.F. Harrington, M. Ayub, A survey of arsenic in foodstuffs on sale in the United Kingdom and imported from Bangladesh, Sci. Total Environ. 337 (2005) 23–30.
[3] M.G.M. Alam, E.T. Snow, A. Tanaka, Arsenic and heavy metal contamination of vegetables grown in Samta village Bangladesh, Sci. Total Environ. 308 (2003) 83–96.
[4] M.L. Kile, E.A. Houseman, C.V. Breton, T. Smith, O. Quamruzzaman, M. Rahman, G. Mahiuddin, D.C. Christiani, Dietary arsenic exposure in Bangladesh, Environ. Health Perspect. 115 (2007) 889–893.
[5] M.C. Freitas, A.M.G. Pacheco, M.A. Bacchi, I. Dionisio, S. Landsberger, J. Braisted, E.A.N. Fernandes, Compton suppression instrumental neutron activation analysis performance in determining trace- and minor-element contents in foodstuff, J. Radioanal. Nucl. Chem. 276 (2008) 149–156.
[6] L. Jarup, M. Berglund, C.G. Elinder, G. Nordberg, M. Vahter, Health effects of cadmium exposure: a review of the literature and a risk estimate, Scand. J. Work Environ. Health 24 (1998) 1–52.
[7] K.P. Mishra, Lead exposure and its impact on immune system: a review, Toxicol. In Vitro 23 (2009) 969–972.
[8] S. Verstraeten, L. Aimo, P. Oteiza, Aluminium and lead: molecular mechanisms of brain toxicity, Arch. Toxicol. 82 (2008) 789–802.
[9] D. Beyersmann, A. Hartwig, Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms, Arch. Toxicol. 82 (2008) 493–512.
[10] P.C. Gupta, C.S. Ray, Epidemiology of betel quid usage, Ann. Acad. Med. Singapore 33 (2004) 31–36.
[11] S.A. Norton, Betel: consumption and consequences, J. Am. Acad. Dermatol. 38 (1998) 81–88.
[12] K.M. McCarty, E.A. Houseman, Q. Quamruzzaman, M. Rahman, G. Mahiuddin, T. Smith, L. Ryan, D.C. Christiani, The impact of diet and betel nut use on skin lesions associated with drinking-water arsenic in Pabna, Bangladesh, Environ. Health Perspect. 114 (2006) 334–340.
[13] J.R. Pilsner, X.H. Liu, H. Ahsan, V. Ilievski, V. Slavkovich, D. Levy, P. Factor-Litvak, J.H. Graziano, M.V. Gamble, Folate deficiency, hyperhomocysteinemia, low urinary creatinine, and hypomethylation of leukocyte DNA are risk factors for arsenic-induced skin lesions, Environ. Health Perspect. 117 (2009) 254–260.
[14] A.L. Lindberg, N. Sohel, M. Rahman, L.A. Persson, M. Vahter, Impact of smoking and chewing tobacco on arsenic-induced skin lesions, Environ. Health Perspect. 118 (2010) 533–538.
[15] FSA, Measurement of the Concentrations of Metals and Other Elements from the 2006 UK Total Diet Study, Food Standard Agency, 2009.
[16] S Ahmed, M. Akter, R. Mahzabeen, S. Sayeed, H. Momtaz, Sayeed, Prevalence of tobacco consumption in a rural community of Bangladesh. MA. & IM-4 MBBS students (batch B), Ibrahim Med. Coll. J. 2 (2008) 58–60.
[17] S.H. Lin, Y.F. Lin, S. Cheema-Dhadli, M.R. Davids, M.L. Halperin, Hypercalcaemia and metabolic alkalosis with betel nut chewing: emphasis on its integrative pathophysiology, Nephrol. Dial. Transplant. 17 (2002) 708–714.
[18] M.T. Wu, Y.C. Lee, C.J. Chen, P.W. Yang, C.J. Lee, D.C. Wu, H.K. Hsu, C.K. Ho, E.L. Kao, J.M. Lee, Risk of betel chewing for oesophageal cancer in Taiwan, Br. J. Cancer 85 (2001) 658–660.
[19] A. Nandakumar, K.T. Thimmasetty, N.M. Sreeramareddy, T.C. Venugopal, Rajanna, A.T. Vinutha, Srinivas, M.K. Bhargava, A population-based case control investigation on cancers of the oral cavity in Bangalore, India, Br. J. Cancer 62 (1990) 847–851.
[20] R. Sankaranarayanan, R.J. Black, R. Swaminathan, D.M. Parkin, An overview of cancer survival in developing countries, IARC Sci. Publ. 145 (1998) 135–173.
[21] A. Auluck, M.P. Rosin, L.W. Zhang, S. Kn, Oral submucous fibrosis, a clinically benign but potentially malignant disease: report of 3 cases and review of the literature, J. Can. Dent. Assoc. 74 (2008) 735–740.
[22] C.W. Vanwyk, I. Stander, A. Padayachee, A.F. Groblerrabie, The areca nut chewing habit and oral squamous-cell carcinoma in South-African Indians—a retrospective study, S. Afr. Med. J. 83 (1993) 425–429.
[23] R. Bedi, Betel-quid and tobacco chewing among the United Kingdom's Bangladeshi community, Br. J. Cancer 74 (1996) S73–S77.
[24] K.V. Shetty, N.W. Johnson, Knowledge, attitudes and beliefs of adult South Asians living in London regarding risk factors and signs for oral cancer, Community Dent. Health 16 (1999) 227–231.
[25] M.V. Gamble, H. Ahsan, X.H. Liu, P. Factor-Litvak, V. Ilievski, V. Slavkovich, F. Parvez, J.H. Graziano, Folate and cobalarnin deficiencies and hyperhomocysteinemia in Bangladesh, Am. J. Clin. Nutr. 81 (2005) 1372–1377.
[26] J.C. Chambers, H. Ireland, E. Thompson, P. Reilly, O.A. Obeid, H. Refsum, P. Ueland, D.A. Lane, J.S. Kooner, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 2448–2452.
[27] S.H. Frisbie, E.J. Mitchell, L.J. Mastera, D.M. Maynard, A.Z. Yusuf, M.Y. Siddiq, R. Ortega, R.K. Dunn, D.S. Westerman, T. Bacquart, B. Sarkar, Public health strategies for western Bangladesh that address arsenic, manganese uranium, and other toxic elements in drinking water, Environ. Health Perspect. 117 (2009) 410–416.
[28] A. Bernard, Cadmium & its adverse effects on human health, Indian J. Med. Res. 128 (2008) 557–564.
[29] M.D. Johnson, N. Kenney, A. Stoica, L. Hilakivi-Clarke, B. Singh, G. Chepko, R. Clarke, P.F. Sholler, A.A. Lirio, C. Foss, R. Reiter, B. Trock, S. Paik, M.B. Martin, Nat. Med. 9 (2003) 1081–1084.
[30] A. Akesson, B. Julin, A. Wolk, Long-term dietary cadmium intake and postmenopausal endometrial cancer incidence: a population-based prospective cohort study, Cancer Res. 68 (2008) 6435–6441.
[31] M. Kippler, B. Lonnerdal, W. Goessler, E.C. Ekstrom, S. El Arifeen, M. Vahter, Cadmium interacts with the transport of essential micronutrients in the mammary gland—a study in rural Bangladeshi women, Toxicology 257 (2009) 64–69.
[32] M. Kile, R. Wright, C. Amarasiriwardena, Q. Quamruzzaman, M. Rahman, G. Mahiuddin, D. Christiani, Maternal and umbilical cord blood levels of arsenic, cadmium, manganese, and lead in rural Bangladesh, Epidemiology 20 (2009) S149–S150.
[33] T.G. Kazi, S.K. Wadhwa, H.I. Afridi, N. Kazi, G.A. Kandhro, J.A. Baig, A.Q. Shah, N.F. Kolachi, M.B. Arain, Interaction of cadmium and zinc in biological samples of smokers and chewing tobacco female mouth cancer patients, J. Hazard. Mater. 176 (2010) 985–991.
[34] G. De Palma, M. Goldoni, S. Catalani, P. Carbognani, D. Poli, P. Mozzoni, O. Acampa, E. Internullo, M. Rusca, P. Apostoli, Metallic elements in pulmonary biopsies from lung cancer and control subjects, Acta Biomed. 79 (Suppl. 1) (2008) 43–51.
[35] R. Kaiser, A.K. Henderson, W.R. Daley, M. Naughton, M.H. Khan, M. Rahman, S. Kieszak, C.H. Rubin, Blood lead levels of primary school children in Dhaka, Bangladesh, Environ. Health Perspect. 109 (2001) 563–566.