

# The Relationship of Dental Extrinsic Stains with the Concentration of Trace Elements in Water Sources in a district of Nepal

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**Purpose:** To assess the relationship of dental extrinsic stains (DES) with levels of trace elements in drinking water sources.

**Materials and Methods:** A cross-sectional study among the population of Madesha VDC. A sample of 551 residents aged between 4 years and above was examined. A questionnaire was prepared to assess the dietary habits, oral hygiene practices, age, period of residency (POR), followed by clinical examination regarding the presence or absence of DES. Water samples were collected and sent for analysis by AAS method for trace elements.

**Results:** The prevalence of DES was 39%. Twenty-eight water samples had Fe levels that exceeded permissible levels and all samples were negative for other trace elements. Stains were associated with POR and levels of Fe in water (P value < 0.001). There was a positive correlation by ward of Fe levels and stain distribution.

**Conclusion:** The results revealed an association between DES with age, POR, and Fe levels in water. The positive correlation between stain distribution by ward and levels of Fe in water indicated the causative role of Fe in stains.

**Key words:** dental extrinsic stains, metallic stains, trace elements, Fe

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The role of trace elements in the etiology of dental caries is well established. The possible role of trace elements in causing extrinsic dental stains has not been studied much. DES are primarily esthetic problems and result from the pigmentation of ordinarily colorless developmental and acquired coatings by chromogenic bacteria, foods and chemicals.

## BACKGROUND

Nepal is a kingdom of high Himalayan Mountains. Geographically the country is divided into mountain, hill and Terai regions. Districts are divided into smaller units called village development committees, and VDCs are sub-divided into wards. People depend on natural springs, tube wells, rivers, canals, ponds and kuwas for domestic, agricultural and irrigation purpose.

A multidisciplinary camp was organized at Madesha VDC, Sunsari District. At the dental OPD, many of the patients complained of discolored teeth, which interfered with esthetics. When a brief history was recorded it revealed that the patients had absolutely no adverse oral habits like smoking, pan chewing, etc., and had rather good oral hygiene hab-

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its. Clinical examination revealed extrinsic staining that was greyish black in color.

Hence the investigators were interested to work in the above area. Consultation with the local authorities revealed that it could be due to water. The possible trace elements in water are Cu, Fe, Mn, Zn, and Ar. Once they exceed their permissible levels all these elements are known to stain laundry, plumbing fixtures, impart undesirable taste, etc., (Park, 1994). Metallic stains are caused by metals and metallic salts, which may be introduced into the oral cavity either by inhaling dust containing metal or through orally administered drugs. These metals combine with acquired dental pellicle to produce a surface stain. Copper dust produces green stain, iron containing medicines cause black deposits, manganese black stain, mercury greenish-black, nickel green, and silver black (Fermin and Michael, 1996). The presence of Cu, Nickel and Fe in the stern layer may result in DES. This is evidenced by workers in the Cu and Ni industries who exhibit green dental stains. Workers at iron industries and people taking Fe supplements exhibit black stains (Faunu, 1983). Nathoo classifies metallic stains as N1-type of discoloration based on chemistry or mechanism of discoloration (Salim, 1997).

Further available literature showed Fe to be one of the causative agents in causing extrinsic dental stains (Tilliss et al, 1992; Warner et al, 1993; Nordbo et al, 1982, 1983). Tilliss et al (1992) in their study showed that Fe in chlorhexidine preparations causes dental stains that had no damaging effects other than that they led to compliance problems for patients.

A few analytical studies carried out by Warner et al (1993) have demonstrated that the use of either chlorhexidine or iron-containing solutions produced heavy staining, and combination elemental analysis of these heavy stains revealed Fe in humans.

Nordbo et al (1983) demonstrated staining to be very severe with Fe solutions and demonstrated a high concentration of Fe in dental pellicle after using chlorhexidine preparation. Ellingsen et al (1982) have reported the presence of sulfhydryl groups in the plaque material that reacts with metals (particularly iron) to form colored sulfides. The source of the sulfur component could be sulfur containing salivary or bacterial proteins. In his study Addy (1995) confirmed that rinses containing chlorhexidine have the potential to cause tooth and tongue staining. All the reviewed literature strongly demonstrated that trace elements especially Fe has a role to play in DES.

## MATERIALS AND METHODS

The investigation was conducted as a cross-sectional study, at Sunsari district, Madesha VDC comprising 9 wards with a population of 6,541. Study Design: Children more than 4 years and elderly dentate persons formed the study group. Exclusion: Edentulous patients, patients with less than 20 teeth, tobacco stains. Criteria for Stains: Extrinsic stains on the tooth surface, which are greyish-black to black in color linear or diffuse, localized or generalized. A structured pre-tested questionnaire and interviews were used to collect information followed by clinical examination. Information on the source of drinking water, diet, oral hygiene practice, POR was collected. Sampling method: It was decided to cover a minimum of 10% of the population at Madesha VDC as this was an exploratory study and the prevalence rate was not available. The total estimated population at Madesha was 6,541 as per Jilla Vivaran Sunsari district for the year 2000 – 2001. Table 1 shows the total sample and the distribution by ward. Our study criteria comprised participants more than 4 years of age therefore 15.5% was deducted from the total sample. Thus the total sample size was 551. Systematic random sampling was followed until the proportionate sample was achieved in each ward. Consent was obtained from the VDC office.

### Method of Examination

People were examined clinically by the same dentist (Pushpanjali) using plane dental mirrors and dental explorers under natural light. Stain was recorded as being present or absent in line with the above criteria. The study was carried out over a period of 10 days.

### Water Sample Collection

Approximately 40 sub-samples of water were collected for analysis as per the instructions given by the laboratory. The samples were collected in fresh and acid preserved 250 ml acid-washed polythene bottles. Water samples were analyzed by automatic absorption spectrophotometer at the Environment and Public Health Organization, Nepal Bureau of Standard and Metrology, Accreditation No. PRA 05/057-8. The equipment used was SOLAR 969 AAS (UK). Samples were analyzed for Cu, Fe and Mn.

**Table 1 Distribution of sample by ward**

Ward No.	Estimated Population*	Sample size
1	941.90	79
2	661.70	56
3	686.72	58
4	545.96	46
5	868.27	73
6	987.99	83
7	693.30	59
8	699.88	59
9	455.18	38
Total	6540.90	551

As per Jilla Vivaran Sunsari, district profile done by branch of Central Bureau of Statistics, INARUWA, for the fiscal year 2000 – 2001.  
\* 15.5% of the population below 4 years of age was excluded.

**Table 2 Fe levels and distribution of stains by ward**

Ward No.	Iron levels*	Prevalence stains
1	3.988	18.6%
2	2.357	12.6%
3	4.343	12.8%
4	1.380	3.75
5	1.630	12.8%
6	1.310	7.6%
7	1.440	8.3%
8	5.318	29.9%
9	1.836	2.8%

$\chi^2 = 175.054$ , DF = 8, P < 0.001  
\* Average of Fe level in samples were taken in each ward.

## RESULTS

Data entry and analysis was done by SPSS package. Pearson correlation, chi-square test and likelihood ratio were performed to establish the association between variables.

The total number of respondents was 551, with an equal ratio of females to males. 84.2% of the participants had been resident in the area for more than 5 years. The prevalence of DES was 39%.

Tube wells were the only common source of drinking water. Diets commonly contained rice, dal, and vegetable. About 67.4% of the participants brushed their teeth with brush and toothpaste, while the remainder used white karani, mud, sticks, and bamboo for brushing.

### Water Sample Testing

Samples were sent for assaying of trace elements using the AAS method. The report showed that out of 40 samples, 28 samples had levels of Fe that exceeded permissible limits (0.3 mg/ltr per WHO guidelines (Park, 1994)) and all the samples were negative for Cu and Mn. Table 2 shows average levels of Fe by ward.

Table 2 and 4 shows that as the level of Fe increases the percentage of people with stains also increases in the respective wards.

The level of Fe was cross tabulated with stains, and the X2 value was 138.865, df = 4, P < 0.001 thus indicating the significant association between stains and Fe levels.

Table 3 shows a significant association between the POR and stains (P < 0.001), and there was a positive correlation between stains, wards and levels of Fe.

Odds ratio is low in cases of participants resident for less than 5 years, i.e. the chances of staining reduces where residency is less than 5 years.

## DISCUSSION

There are quite a few studies linking the role of trace elements in causing DES.

In the reviewed literature Mn, Ni, Ag, Fe, Cu are known to cause DES. The sources of these elements are either dust or medical preparations. Their possible role with their presence in H<sub>2</sub>O is not reported in the available literature. This study showed the presence of Fe to have exceeded permissible levels in 28 water samples out of 40. Although the reviewed literature related the role of Fe in stains it was mainly in the form of dust, medicated mouth rinses, or liquid iron preparations. Only the trace elements Cu, Fe, and Mn were assayed because these are known causes of DES.

**Table 3 Association between period of residency and stains**

POR	STAINS
Less than 5 years	25.9%
More than 5 years	74.1%
$\chi^2 = 47.072$ , $df = 1$ , ( $P < .001$ )	

Few studies have visually estimated the stains according to standard criteria but this approach was not possible since the present study was a prevalence study with the primary aim of assessing the presence or absence of stains, rather than the intensity and extension like other clinical studies (Dolles et al, 1979).

Iron compounds generally stain teeth black, and iron sulfide is found as an extrinsic staining material in plaque. In ferrous form iron is found at a concentration of up to several mg/lts without causing discoloration or turbidity. On exposure to the atmosphere it oxidizes to ferric form and adds an objectionable reddish-brown color to water. Sulfur is a natural component of plaque; probably Fe reacts with this sulfur and forms ferrous sulfide, which produces the black color (Ellingsen et al, 1982).

The results of this study showed: firstly, that Fe content in the water samples exceeded permissible levels, and secondly confirmed the probable role of Fe in causing DES.

The significant association between POR and age with staining probably accounts for the duration of exposure. The study also demonstrated the significant association between staining and Fe levels in water. A positive correlation existed between staining and iron levels by ward. Table 2 shows ward numbers 1, 2, 3 and 8 had Fe levels that exceeded permissible levels, and more people to have stains in these wards compared to ward numbers 4, 5, 6, 7 and 9. In wards where the Fe level was 1 mg or less, there was a correspondingly higher percentage of people without staining than those with stains.

**Table 4 Levels of Fe and percentage of people with stains**

*Fe levels in Drinking water	Stains
1	7.0%
2	12.6%
3	18.6%
4	12.8%
5	20.9%
As level of Fe increased, percentage of people with stains also increased. *Fe levels are expressed as equal to, or more than.	

In conclusion, based on the available resources we were able to confirm the probable causative role of Fe in causing DES. Although these stains are harmless other than from an esthetic perspective, a more important factor is the role of diet and higher levels of Fe in water consumed with the possible consequences of hemochromatosis and hemosiderosis (Murray et al, 1993). We also would like to stress the importance of the geographic location with reference to participants complaining of black stains on their teeth, and also educate patients regarding maintenance of good oral hygiene.

### Recommendations

The exact mechanism of staining needs to be studied, and the interaction with other agents should be considered. More detailed clinical studies pertaining to scrapings of stained material from the tooth surface is required to confirm the presence of Fe.

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