

Dental fluorosis and fluoride in Willoq and Cuzco: assumptions and reality in Peru

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Abstract

Background: Dental fluorosis (DF) is a dental disease characterized by visual imperfections, staining, and opacity. DF is caused by superoptimal levels of the common fluoride (F) ion in consumed water sources. Natural levels of F fluctuate wildly based upon environmental, industrial, and geologic factors. Current global DF and F predictions based on location are still largely preliminary, especially in the developing world.

Objective: To assess the (DF) status of the indigenous community of Willoq and a boys' orphanage in Cuzco, both located in the Sacred Valley region of Peru; to determine the F concentration in the main sources of consumed water in these two locations and in the city of Arequipa; and to compare the F levels and DF status against previous predictions.

Materials and Methods: Individuals from Willoq and Cuzco, aged 8–69 years were examined for DF based on the Dean's Index. In each community, $n = 29$ for a total of $n = 58$. Population statistics were represented through the calculated Community Fluorosis Index (CFI). Fluoride concentrations were measured using a F ion selective electrode following EPA method 340.2.

Result: The prevalence of DF in the selected groups from Willoq and Cuzco is 7% and 29%, with CFI values of 0.03 ± 0.13 and 0.60 ± 1.14 , respectively. Willoq and Cuzco F concentrations in the water sources were 0.285 and 0.151 mg/L, respectively, while Arequipa water had a F concentration of 0.193 mg/L.

Conclusion: There is a low prevalence of DF in Willoq, which corresponds to negligible water concentrations of F. Cuzco has higher levels of DF at similarly low F concentrations, a result that suggests additional F sources are not accounted for in this analysis. Arequipa's low F concentration and the nonzero DF in Peru are contrary to the previous predictions and call attention to a need for better and more refined DF and F predictions.

KEY WORDS: Dental fluorosis, fluoride, Peru

Introduction

In day-to-day life, the teeth are exposed to a wide variety of substances from many sources. Fluoride (F⁻, also written as F in some literature), the anionic form of fluorine, is one such

element. In areas of the world where the primary water sources are from the ground, the range of groundwater F concentrations vary based on factors such as geology, agricultural cultivation intensity, level of industrialization, and annual precipitation.^[1] At optimal concentrations, F provides benefits to the dentation of all individuals; indeed, public drinking water fluoridation in developed countries is cited as one of the top ten public health victories of the twentieth century.^[2] Fluoride is considered to be a beneficial nutrient at optimal levels, is found throughout the body in trace amounts, and is important for the health and structure of the bone and teeth.^[3] Fluorine's electronegativity creates a strong interaction with calcium. Thus, more than 99% of F in the body is found in the bone and teeth.^[3]

Fluoride has a number of protective effects on the dentation. Fluoride produces a cariostatic effect (reducing risk of

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dental caries) in all individuals by its presence in oral fluids such as saliva and dental plaque. In this context, it reduces the acidogenic ability of oral bacteria and increases the rate of remineralization during an acidogenic challenge.^[4,5] Fluoride has an effect as well on individuals during the pre-eruptive development of permanent dentation. The maturation of the crowns of the anterior permanent dentation is complete by the age of 8 years.^[6] During enamel maturation, F is integrated into the calcium-rich enamel crystals to form fluorhydroxyapatite [various crystallographic substitutions of OH⁻ by F⁻ with the formulas Ca₁₀(PO₄)₆F_xOH_{2-x}], which is less soluble in acidic environments than is the F-absent hydroxyapatite, Ca₁₀(PO₄)₆(OH)₂.^[5]

Excess fluoride may cause two well-studied diseases: skeletal fluorosis (SF) and dental fluorosis. DF is an undesired tooth mottling, which results from superoptimal levels of F during enamel formation. Fluoride in this context provides both benefit and harmful effects: DF is considered as an unfortunate effect of the F, which simultaneously provides the abovementioned cariostatic effects. DF is primarily viewed as a cosmetic problem, which may affect the dental esthetics and psychological status of the individual but causes no adverse consequence to the dental function.^[7-9] There has been a debate on the optimal concentration of consumed F in water, but 1 mg/L is considered both effective and safe.^[10-12] The World Health Organization (WHO) considers 0.8–1.2 mg/L to be the range in which maximum benefits are expected, with 1.5 mg/L as the global acceptable upper limit for F concentration.^[13,14]

Although evidence suggests lower concentrations may give rise to DF, generally significant DF does not occur at levels less than 1.5–2.0 mg/L, unless other intake routes are sources of F. These sources may include high-fluoride coal burning, food, or tea.^[11,15-17]

SF is a more serious disease, affecting the skeletal system and presenting symptomatically as stiffness and pain in the joints. Being difficult to distinguish symptomatically from certain types of arthritis, SF may be radiographically diagnosed by increased bone density with reduced strength or by F in bone ash concentration above normal levels. Crippling SF is most predominantly a concern at F concentrations above 10 mg/L, although this has been contested by claims of significant SF found at lower levels, especially when diagnosis is based on clinical instead of radiological criteria.^[16,18,19]

Because many countries standardize their public utility water F concentrations according to international guidelines, the majority of DF and SF cases occur in areas where the consumed water is collected from naturally occurring sources. In particular, China, India, and parts of Africa have had high levels of groundwater F and well-documented problems with DF and SF.^[20-23]

The abovementioned regions are well-studied in terms of their F levels and corresponding DF prevalence. However, other regions of the globe are less studied, and there is a need for more research on both these fronts. Some groups have tried to chart and predict F concentration levels in various countries, including Peru, based on factors such as the

underlying geologic environment or agricultural cultivation intensity, level of industrialization, and annual precipitation.^[24,25] A global disease burden estimate has been created for DF by the country, but this is only a preliminary investigation into the subject.^[1] In Figure 1, the mountainous Andean region of mid to southern Peru is predicted to be at a medium risk of greater than 1.5 mg/L fluoride, based on geologic data and on its arid/semi-arid environmental status.^[24,25] Previous studies have examined populations in areas not predicted by Figure 1 to have above WHO-recommended levels of DF.^[26] In addition, one of the first preliminary disease burden models predicted no DF to be present in Peru^[1]; this stands in contrast to the national data collected by the Peruvian government, which indicates 10.1% national DF prevalence.^[27] Independent investigations also find DF in Peru.^[26] Currently, predictions of DF do not align with the reality in Peru. Thus, this pilot study endeavors to take preliminary F and DF data for two communities in Peru, a boys' orphanage in Cuzco and the indigenous community of Willoq [Figure 2], which are not predicted to have a significant F nor DF based on the aforementioned model. These two communities also obtain drinking water primarily from one or two sources. The F concentration of a community water source in Arequipa was also tested. Arequipa is a city that falls inside the area with the medium potential for above WHO-recommended levels of F in groundwater.

Materials and Methods

Study Design

This pilot study was conducted in January 2014 among individuals aged 8–69 years in two communities in Peru: Willoq, a traditional Quechua community located in the Sacred Valley, and Maria Salome Ferro, a state- and church-run boys' orphanage in Cuzco. These communities rely on a few and known sources for drinking and cooking water. Volunteers recorded demographic data; volunteer American dentists assessed and recorded the fluorosis status of individuals during the course of a complete intra-oral evaluation. Water samples taken from the community water sources were collected for analysis. In addition, water samples were taken from a church compound in Arequipa. No DF data were taken from this site.

Clinical Evaluations

American-trained volunteer dentists completed clinical intra-oral evaluations. DF status was graded according to the often-used Dean's Index [Table 1].^[28] Providers were provided with Table 1 before and during their examinations.

Individuals who satisfied the following criteria were included in the study:

1. Individuals from within the local community who were assumed to use the sampled water sources as the primary water source.
2. Individuals aged 8 years or older in order to ensure the presence of erupted permanent dentition.^[29]

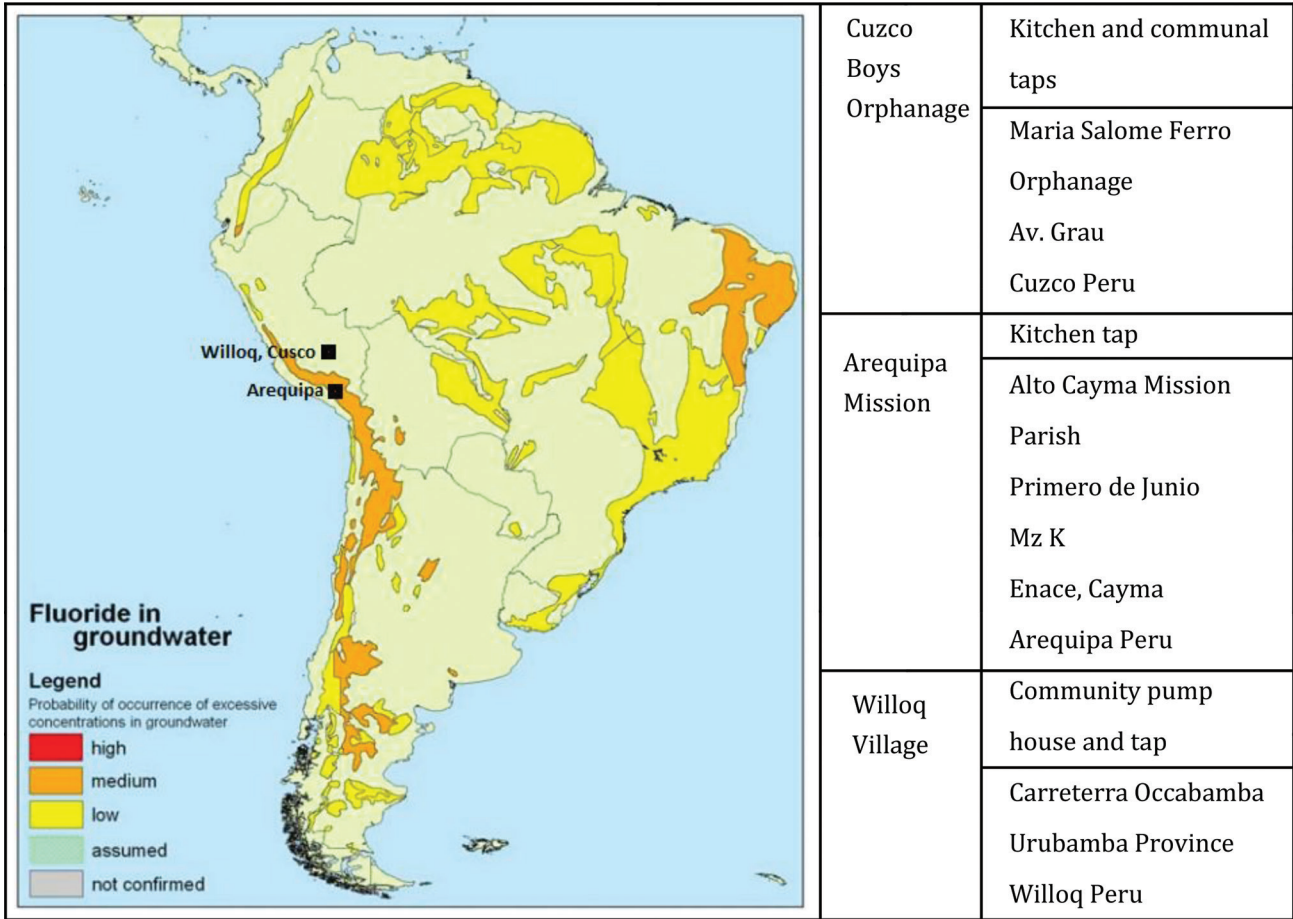


Figure 1: Geographic prediction map of probability of greater than 1.5 mg/L F concentration in groundwater in South America and the locations of the three sites in Peru^[24].



Figure 2: Expanded geographic relation of Cuzco and Willoq in the Sacred Valley region of Peru. Map data: Google.

- Individuals who had previously agreed to intra-oral examination and free dental work from the volunteer dentists.
- Individuals who were cooperative.

Data were taken from anonymized intra-oral evaluation forms on which was recorded, among other data, the visually determined DF status of the individual and their demographic information. Participants had previously consented to recorded intra-oral evaluation and a wide variety of dental work completed by volunteer dentists. Permission and oversight was obtained from St. Olaf College's Institutional Review Board, before this work.

Collection and Analysis of Water Samples

Water was collected at the main sources of drinking and cooking water use in the community. Ten milliliter polyethylene terephthalate (PET) bottles were rinsed with deionized water and dried before transportation to the collection point. Samples were taken from the community water tap and a

Table 1: Dental fluorosis categorization

Dean's index score	CFI scores	Criteria
None/normal	0	Smooth, glossy, pale creamy-white translucent surface
Questionable	0.5	Slight aberrations in translucency, ranging from a few white flecks to occasional white spots
Very mild	1	Small opaque paper white areas scattered irregularly or streaked over the tooth surface; involves less than 25% of tooth surface
Mild	2	White opaque areas more extensive but do not involve as much as 50% of tooth
Moderate	3	All enamel surfaces of the teeth affected; surfaces subject to attrition show marked wear; brown stain frequently a disfiguring feature
Severe	4	All enamel surfaces affected and hypoplasia so marked that general form of tooth may be affected; major diagnostic sign of this classification is the discrete or confluent pitting; brown stains are widespread; teeth often present a corroded-like appearance

communal water pump house in Willoq, which piped water to the higher farms and homes in the mountainous town. At the Cuzco orphanage site, samples were taken from the community drinking tap and from the communal kitchen. Water from the sample sources was allowed to run for 10 s. Bottles were then rinsed with the source water, and the water was discarded. Bottles were then filled with the sample and capped.

Water samples were analyzed for F concentration in the laboratories of the Department of Chemistry, St. Olaf College, Northfield, MN, following the procedure outlined in EPA 340.2 using an Orion Fluoride Combination Electrode (9609BNWP) ion selective electrode (ISE).^[30] In this method, equal volumes of sample and buffer solution were mixed, and the fluoride sensitive electrode was used to measure the potential difference. Buffer solution contained 1,2-diaminocyclohexanetetraacetic acid (CDTA) (Alfa Aesar, 98%), a strong chelating agent that counteracts any effects of Al^{3+} , Fe^{3+} , and Si^{4+} ; glacial acetic acid and sodium hydroxide to create a buffer solution of pH 5.0–5.5; and sodium chloride. Potentiometric results from ISE were recorded in spreadsheet software. Standards of sodium fluoride (Acros Organics, 99%) were created at the following concentrations: 0, 0.02, 0.05, 0.08, 0.1, 0.12, 0.15, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, and 10.0 mg/L.

Result

Population

Two populations were included in this pilot study for a total of $n=58$, where both Willoq and Cuzco had a total of $n=29$. In Willoq, the study group comprised nine (31%) male subjects and 20 (69%) female subjects. The mean age of the Willoq population was 29.6 years, with a standard deviation of 18.0 years. In Cuzco, the study group comprised 29 male subjects (100%). In this boys' orphanage population, the mean age was 12.5 years, with a standard deviation of 2.6 years. Figure 3 illustrates the age distribution of the two communities.

The sampled population of the Willoq community is composed of both male subjects and female subjects from

a wide age range. Thus, the age distribution of the Cuzco sample is much younger than that of Willoq [Figure 3]. The Willoq sample showed a “negative public health significance” level of DF^[31] [Table 2], with a Community Fluorosis Index (CFI) = 0.03 ± 0.13 . CFI was calculated by averaging CFI values [Table 1] corresponding to each individual. Only two of the individuals tested were found to have DF, and none showed higher than a “questionable” rating on the Dean's Fluorosis Index [Table 2]. Cuzco showed a higher community sampled level of DF with a CFI = 0.60 ± 1.14 , a value at the highest cutoff for the “borderline” public health significance CFI classification.^[31] In both the communities, the majority of the tested individuals did not present DF. In Cuzco, there were not only higher levels of the milder forms of DF but also numerous examples of the highest classifications possible, with 24% of the tested population having greater than “questionable” classifications on the Dean's Fluorosis Index [Figure 4].

To compare the DF status of the Willoq and Cuzco communities, a Wilcoxon–Mann–Whitney test was performed. This test was chosen owing to the ranked nature of the Dean's Index. There is an evidence that the fluorosis levels are statistically significantly different between the two communities ($p=0.0068$). Statistical analysis was completed using RStudio (RStudio, version 0.97.318; RStudio, Inc., Boston, MA).

Fluoride Concentrations

The F concentrations of the community drinking water for the three communities were measured at representative communal drinking sources. In Willoq, a mean of 0.285 mg/L F was found from the two sources: the community water pump house and the communal tap in the village center. In the Cuzco orphanage, a mean of 0.151 mg/L F was found from the samples taken from the two locations: the communal tap in the courtyard [Figure 5] and the kitchen water used to prepare food for all the individuals were tested.

In Arequipa, a value of 0.193 mg/L F was found from the community kitchen at the Alto Cayma Mission Parish, Arequipa. It is noted that this is the single location tested where predictions indicate a moderate probability of greater

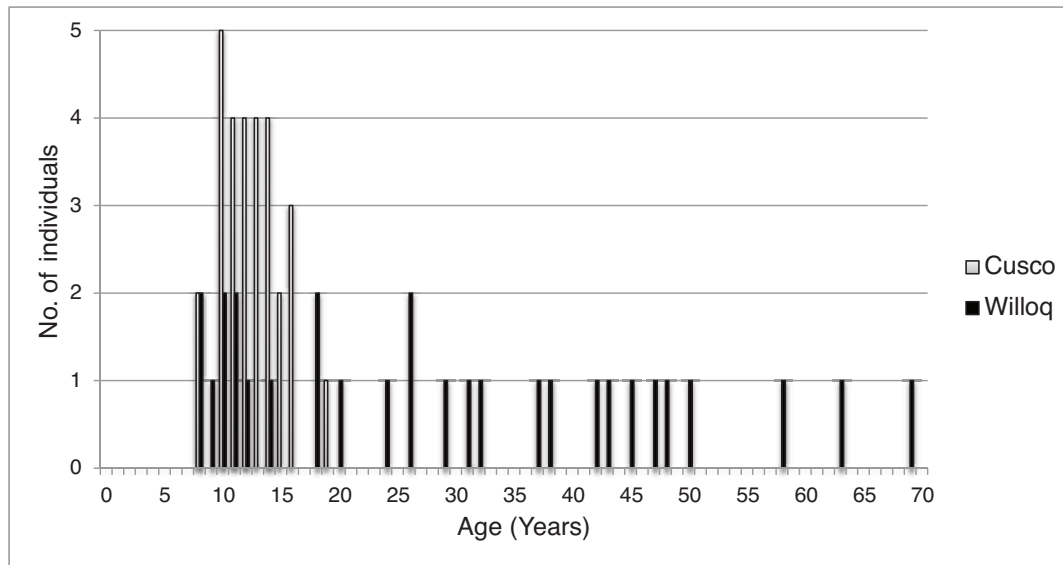


Figure 3: Age distribution in Cuzco and Willoq.

Table 2: Dental fluorosis status by location

Dean's Fluorosis Index	Location	
	Willoq, n (%)	Cuzco, n (%)
Normal	27 (93)	19 (66)
Questionable	2 (7)	3 (10)
Very mild	0 (0)	3 (10)
Mild	0 (0)	0 (0)
Moderate	0 (0)	3 (10)
Severe	0 (0)	1 (3)
No. examined	29 (100)	29 (100)
No. with DF	2 (7)	10 (34)
No. with > "questionable" DF	0 (0)	7 (24)
CFI	0.03 ± 0.13	0.60 ± 1.14
Median Dean's Fluorosis Index	0	0

than 1.5 mg/L F concentrations.^[24,25] Individual tested concentrations for the three locations are found in Table 3.

Discussion

This study was designed to serve as a preliminary investigation to explore questions of DF in Peru. The two populations were chosen because of their status as ostensibly isolated communities. In the case of Willoq, the population is almost exclusively indigenous and speaks the native Quechua, and individuals are likely to either have lived in Willoq or the surrounding area for life. This, in conjunction with their communal and natural water source, the F levels of which we assume to have been constant during the lifetimes of those sampled, creates conditions for a relatively homogenous and pure community to sample.

Because of the nature of the project and location, it was not possible in Willoq to create a significant sample of children close in age to one another; thus, a wide range of ages were sampled (mean age = 29.6 years). Studies worldwide commonly use primary school children as their sampled population.^[21,26,32] However, F exhibits protective effects past the eruption of permanent teeth well into adulthood.^[33] A study by Rwenyonyi et al.^[34] tested two populations, one which consumed water at 2.5 mg/L and the other at 0.5 mg/L. DF classification increased over time only in the higher F group; the lower F group's DF status was constant, as they tested in the age range of 10–14 years. Thus, because F concentration in Willoq is very low, 0.285 mg/L, and there is almost a complete lack of DF in Willoq, it is reasonable to assume the DF descriptive statistics for this sample are age independent and the population statistics can be reasonably compared with other studies. Fluoride concentrations are below 0.3 mg/L and, thus, in the range of what others in the literature consider negligible.^[35]

In the Cuzco community, the age of the sample was much younger (mean age = 12.5 years) and exclusively male subjects; the CFI value for the community was at the highest end of the borderline classification. Samples were taken from a locked boys' orphanage. Since the boys ate and drank exclusively at this location when they lived there, it was assumed that their DF status was tied to their F consumption from these sources. While this seems at first like a reasonable assumption owing to the isolated nature of the community and homogeneity of the population, this assumption becomes quickly problematic for three reasons. First, the boys arrived at the orphanage at some unknown point after the age of five years, because children are not accepted until this age. However, children are especially susceptible to DF of the

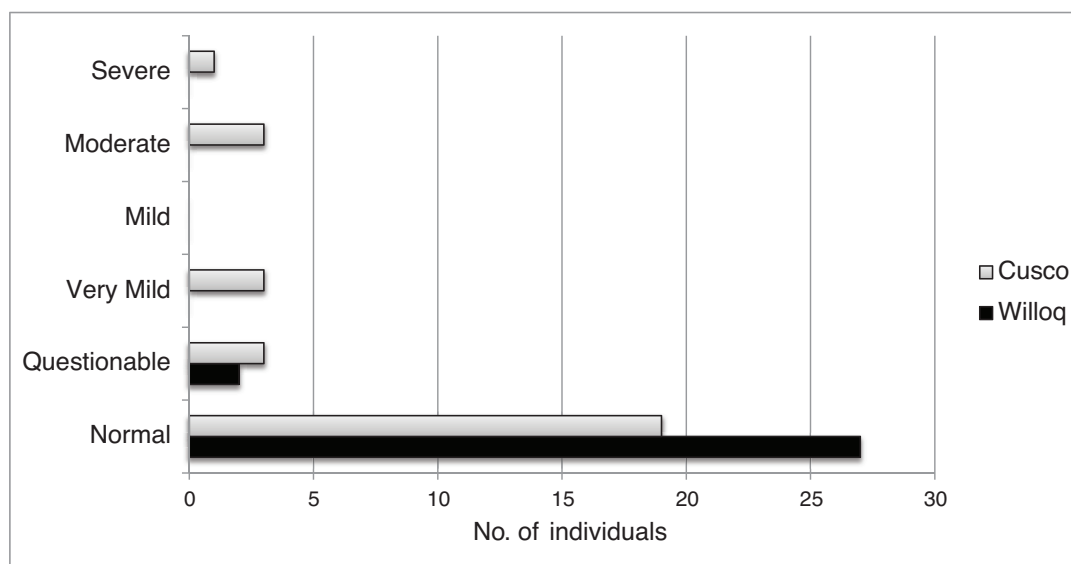


Figure 4: Dental fluorosis in Cuzco and Willoq.



Figure 5: Community tap, Maria Salome Ferro Orphanage courtyard, Cuzco, Peru. Photograph courtesy of author.

secondary upper incisor teeth between 22 and 26 months of age.^[16] Depending on the age of their admission into the orphanage, they all would have had varying levels of F from outside sources during their first years of life. Second, owing to the nature of orphanages in Peru, the majority of these children are not orphans but rather unable to be resource-supported by their birth family. During portions of the year where extra help is needed, a family may take their child home again for a time, returning them to the orphanage

when resources again become scarce (e-mail to David Brendan Johnson from Professor Theodore Johnson, PhD, December 11, 2014). During this time, they would consume F from unidentified and untested sources. In addition, it is not known what proportion of the sample may participate or what amount of time a child may spend away from the orphanage. Finally, there is a high rate of turnover from year to year among the children.

The F concentration in Cuzco was 0.151 mg/L, which as previously stated was considered a negligible concentration. Thus, the most reasonable explanation for higher levels of DF is the one in which the children with DF in the Cuzco sample, and especially those with moderate and severe classifications, received the causal F intake from sources that are not the communal sampled tap or kitchen. While this again implies the initial and, at first glance, sensible assumptions about the single F source nature of this community were perhaps not applicable for the above-cited reasons, it also implies other important conclusions. First, that the DF level of Cuzco, and thus Peru as a whole, is not zero, contradicting predictions that the DF burden in Peru is zero. Thus, these results call for more refined models in DF disease burden prediction. Second, the prediction models have called for more data regarding water F concentrations from around the world, and this work is one step toward future study. Finally, the data imply that the DF and F of the region from which these individuals are taken are necessarily easily correlated. Again, examination of the data leads to the hypothesis that the status of the individuals with higher-severity DF may be primarily a result of alternate risk factors such as not-sampled groundwater F concentration, nutritional status, altitude, and other fluoride exposures.^[36,37] This lesson should be taken into consideration in future DF work; researchers often sample a

Table 3: Fluoride concentrations by location

Location	Source	Experimental F concentration (mg/L)	Mean F concentration (mg/L)
Cuzco	Community tap	0.227	0.151
	Kitchen tap	0.074	
Willoq	Community pump house	0.275	0.285
	Community tap	0.304	
	Duplicate of community tap	0.286	
Arequipa	Kitchen tap	0.193	0.193

similar textbook case of a supposedly homogenous school-age group from one location and assume their DF status is directly and easily linked to local F levels. As this work shows, two communities with similar initial assumptions and similarly negligible levels of F may present statistically significantly different levels of DF. Thus, this is likely a result of the failure of the applicability of the assumptions to one of the communities.

The Arequipa location showed an F concentration of 0.193 mg/L at the tested location. Arequipa is in the area predicted to have a “medium” risk of greater than 1.5 mg/L F concentration, while Willoq and Cuzco are not.^[24,25] Arequipa is thus predicted to have a higher concentration. Because this was not observed, a future study may examine different locations in the city and nearby medium risk zones in order to verify the validity of the predictions. Furthermore, additional externally validated DF statistics would be useful in order to gauge the extent to which DF is a problem in this community. As it currently stands, the Arequipa concentration calls into question the validity of the cited groundwater F predictions. Future work may investigate the possibility of unique considerations, which may apply in the local region, Peru, or the continent as a whole, which may modify or influence model predictions. Future work may also investigate further the intricacies of the stories of the Cuzco children and how this influences their dietary consumption. This may inform how the assumptions made in many studies, which purport to determine the DF prevalence, may need modification. Finally, this work suggests that, in all three locations, public policy to standardize, thus in this case to increase, the F concentration to optimal values should be considered in order to promote caries prevention and improve public oral health.

Conclusion

The mean levels of F in the water sources tested are considered negligible. DF does not meaningfully exist in isolated Willoq but does exist in the boys’ orphanage in Cuzco, shown by its borderline CFI value in the standard Dean’s classification. Population level indices such as the CFI may miss important information such as the extreme distribution of DF in Cuzco, which leads to the hypothesis that the more severe cases received their F intake at other times and places than were tested, highlighting the importance of validating initial future assumptions. Arequipa’s tested level of F and the confirmation that the disease burden of DF was nonzero, both

in contrast to the previous projections, highlight the need for more accurate and powerful predictions.

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