Investigating impacts of natural minerals in drinking water on skeletal development and dental health - a cross-sectional study

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Abstract. Drinking water works as an important supplementary source to maintain human health. To find out the relationship between minerals concentration and skeletal development and dental health for school children, we investigated three village primary schools located in northwest China where drinking water minerals varied. It was found out that Ca, Mg, F and Sr ranged in 31.8-90.1 mg/L, 10.3-58.1 mg/L, 0.6-2.8 mg/L and 0.64-14.2 mg/L respectively in local drinking water. Students’ height aged at 11 and 12 in low Sr and normal Ca group was found significantly lower; students’ height aged at 13 was higher in the high Sr and low Ca group. The students in low Sr, normal Ca, low Mg and F group was identified with the most incidence of delayed skeletal development, and the least incidence for dental fluorosis and dental caries. The results implied Sr did not damage skeletal health at concentration of 14.2 mg/L in drinking water, nor play as a protective factor for dental caries, but it could be risky for dental fluorosis when F existed in drinking water. Mg at concentration of 44 mg/L in drinking water was not proven effectively to protect children from dental caries.

1. Introduction
Adolescent students are in a critical time of bone growth and mineral acquisition [1-2]. A sufficient supply of minerals, including Ca, Mg, F and Sr are essential for their development. There are three paths of chemical elements exposed to the human organism: ingestion, inhalation, and dermal contact [3]. Ingestion of food and water is deemed to be the main exposure route of mineral compounds in a natural, unpolluted environment. Chemical elements dissolved in drinking water mainly exist as the iron form, and their bioavailability are similar to that from food. When people keep consuming drinking water from the same source, and the intake of minerals from drink water route has remained unchanged for great lengths of time or even a lifetime until they move to another place. Here, it is of critical importance to note that in fact, schoolchildren in China spend nearly ten hours per day at school. Water consumption at school plays a significant role and accounts for a large proportion in total water use, all aspects of drinking water quality should meet the requirements presenting no harm to people who keep drinking it in their lifetime. Unlike microbial aspect, some inorganic chemical elements from drinking water are indispensable for human health, especially for school students who are fast growing and in the restoration period.

Chemical composition concerning human health in the environment can be considered as a
“medicine” or a “poison” depending on dose-response relationship. Drinking water quality indexes are often regulated “contaminants” to protect health. The European Food Safety Authority (EFSA) established F upper limit of exposure to 1.5 mg/L [4]. This value limit is confirmed also by World Health Organization drinking water guideline [5]. The Environmental Protection Agency (EPA) announced a preliminary regulatory determination for Sr in drinking water and set the health reference level (HRL) for Sr(II) at 1500 µg/L [6].

However, some essential mineral compounds including Ca and Mg are not being listed in drinking water quality guideline, such as the World Health Organization (WHO) drinking water guidelines [5] although adequate Ca intake is essential for achieving peak bone mass and subsequent prevention of osteoporosis [7-8]. Conversely, there are many publications documenting incidence for osteoporosis in humans as well as skeletal degradation in animal models with a deficit of Ca and Mg in drinking water [9-10]. Moreover, Ca, as the main component of teeth, is beneficial to prevent dental caries, and inefficient Ca intake can cause such disease. According to the literature, it’s pointed out that dental caries often arises in childhood as aggressive tooth decay although people are susceptible to the disease throughout life [11-12].

Edzwald JK states [13], although water is not the only source of F, drinking water is a significant and major source of exposure of dietary intake for most people. Prior research has suggested proper concentration of F ingestion can reduce the incidence of dental caries or decay and promote bone mineralization [14]. There is a u-shaped dose response relationship between the intake of fluoride in drinking water and the prevalence of dental caries and dental fluorosis [15]. The lack of F exposure can cause dental caries disease. In other cases, high levels of F has shown some toxic impacts on long-term exposure in water, for example, osteoporosis [16], periodontal disease [17], dental fluorosis [15] and decreased intelligence [18-20] in children. Studies [21] have shown that Ca and Mg might have the antagonistic effect on fluorosis, however the impact of each factor is not equal.

The main exposure routes to strontium are drinking water and food with drinking water being the predominant source. It is similar to calcium, as the main component of bones and teeth, strontium can put a link to the formation of human bones. Deficiency can cause tooth decay, and excess concentration exposure can lead to bone growth and development too fast, such as joint and bone deformation thick, weak pain, muscle atrophy and anemia [22]. Moreover, Hirata found that strontium could replace calcium ion in the form of calcium hydroxide phosphate and stimulate reaction between fluoride and hydroxyl [23]. Animal experiment found that continue exposure may affect bone development following ingestion of high doses, and young rats were more vulnerable than adult rats. Although the data on human health impacts exposure to strontium is very limited, a detailed epidemiological investigation indicates that a high level of strontium in experimental animals may pertain to human [21]. It is worth of mentioning that population who are more vulnerable to adverse effects of Sr contamination are those who absorb strontium at high rates as well as those who live in poverty and have limited access to get nutritious food, most of them are children. Because of low protein and Ca diet, it will be more easily for Sr to replace Ca in skeletal development.

To investigate the effect of low-mineral or high-mineral on the physical development and dental health of adolescent students, we selected students aged from 10 to 13 in 3 rural schools in northwest China, examined the concentrations of chemical elements in drinking water, measured and diagnosed the children's physical developmental and dental health status, and tried to identify the risky level and possible interaction of these chemical compounds exposure from drinking water.

2. Literature review

The EPA set the health reference level for Sr(II), listed at 0.5-1.5 mg/L which comes from a preliminary regulatory determination for Sr in drinking water [6]. Low Sr is beneficial for bones. Studies showed that low Sr can increase the number of bone cells and enhance bone cell replication and bone formation in vitro [24]. while high concentration of Sr can cause abnormal bone mineral metabolism. Rats experiment indicated that a high dietary intake of Sr induced the development of rickets for the increasing incidence of osteoporosis and bone sialoprotein [25]. Moreover, bone
mineralization could be more likely occurred in young animals than in the older ones [26-27]. Many epidemiological investigations and animal experiments have demonstrated that Sr has a critical effect on dental health, and found that Sr concentration in high-caries communities was significant lower than in low-caries communities [28-31]. These findings suggested that there was a significant negative relationship between dental caries and Sr.

World Health Organization recommend F should not exceed 1.5 mg/L in drinking water in the Guidelines for Drinking Water Quality [4]. In a study in America, there was a significant side effect between mild-to-moderate dental fluorosis and F [32]. A study pertaining to the relationship between serum F levels in 8-12 year-old children and endemic fluorosis found a significant positive correlation between high-F drinking water and the prevalence of dental and skeletal fluorosis [33]. In a case conducted in Jiangsu, China, on the effects of F on 8-13 aged students, there was a u-shaped dose response relationship between the total intake of fluoride in drinking water and the prevalence of dental caries [34]. A survey of 8,266 people over 50 years old, which was conducted in China, showed that long term F exposure from drinking water can increase the risk of overall fracture as well as hip fracture [35].

It is widely recognized that Ca and Mg play important roles in bone growth and development in children and adolescents, and also the maintenance of bone mineral loss in postmenopausal women [36-37]. One study conducted in 45 early postmenopausal young women with one year of consumption of either Ca-rich or low-content Ca water revealed that low-Ca group had a significant decrease in bone mineral density (BMD) of the wrist [38]. In female rats’ experiments, low dietary Ca and Mg intake including drinking water, can leads to decreased bone formation [10]. A survey sampled 225 women, they found that regularly using Ca-rich drinking water can improve the average of vertebral BMD [39-40]. Similar results were showed in a large sample of 4434 women over 75 years old, an increase of 100mg per day in Ca from drinking water was associated with a 0.5% increase in femoral neck BMD was observed [41].

3. Study objectives

Our study was designed to do preliminary research on the interaction effect of minerals in drinking water on school student’s skeletal development by measuring and comparing of height, bone age and incidence of dental fluorosis and caries among differentiated drinking water minerals exposure groups. As nutrients intake from drinking water is one of the important ways for population especially adolescent health to obtain sufficient and effective nutrients intake, this study was expected to provide preliminary population research proof to help determining the lower or upper limit of Sr, Ca and Mg which have not been included in current drinking water quality standard.

4. Materials and methods

4.1. Study area description

We chose one county in Shanxi in northwest China as our research location. As children are more sensitive to mineral intake than adults, we selected 3 primary schools in this county with different drinking water source and employed students aged 10-13 as our targeting population. School 1 (S1) and school 2 (S2) used groundwater as drinking water source and school 3 (S3) used rainwater collection. The location and distribution of the researched schools are shown in the figure 1. To control confounding factors, the three schools located in neighboring area with the similar average income per capita and dietary habit.
4.2. Water sample collection and testing

Water samples were collected from the operational in use taps in the campus to test Ca, Mg, F and Sr. All students in these three schools were consuming water that we collected as their only source for drinking, washing and food preparing during their stay in campus. We collected water samples from taps in the school campus, each sample had a paralleling sample. We transported the samples to local laboratory within 2 hours at most and tested immediately. The reported water quality test result was the average of two paralleling samples testing result. Ca and Mg was tested by volumetric method, F was test by ion selective electrode method, and Sr was tested by atomic absorption method respectively, which are recommend in national laboratory testing guidelines, Methods for Examination of Natural Mineral Drinking Water Quality and Standard Examination Methods for Drinking Water Quality.

4.3. Physical examination of selected students

Physical examination of all students for their height, dental caries and dental fluorosis was conducted. Physical height examination followed the related criterion, requesting students stand straightly shoe-off and measure the vertical length from top of skull to the floor. Among all students, we randomly select at least 30% students from that attended the study to undertake palm x-ray examination, evaluated as The Maturity and Evaluation Method of Wrist Bone for Chinese Teenagers. Dental fluorosis and dental caries were diagnosed, following Dean’s index [42] and guideline of oral health issued by World Health Organization [43] respectively. 

Two indicators were used to describe students’ dental fluorosis, which is dental fluorosis prevalence and medium or severe dental fluorosis prevalence. Following indicators were considered to analyze the dental fluorosis.

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4.3.1. \text{Dental fluorosis prevalence (DFP)} = \frac{\text{case number diagnosed as dental fluorosis}}{\text{all enrolled cases}} \times 100\%
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4.3.2. \text{Medium and sever dental fluorosis prevalence (SDFP)} = \frac{\text{number of case diagnosed as III degree and severer dental fluorosis}}{\text{all enrolled cases number}} \times 100\%
\]
4.3.3. Dental caries prevalence (DCP) = \frac{\text{cases diagnosed as dental caries}}{\text{all enrolled cases number}} \times 100\%

4.3.4. Average number of caries tooth in one group (ANCT) = \frac{\text{accumulated number of caries tooth found in one group}}{\text{cases number diagnosed as caries tooth}} \times 100\%

4.4. Statistics analysis
SPSS 18.0 software was used for data analyze, and t-test for difference of height and average number of caries tooth among groups, \(\chi^2\)-test for difference of bone development and prevalence of dental fluorosis and caries among groups.

5. Results and discussion
5.1. Results

5.1.1. Water quality. Water quality test results are showed in figure 2. Ca, Mg, F, and Sr ranged in 31.8-90.1 mg/L, 10.3-58.1 mg/L, 0.6-2.8 mg/L, and 0.64-14.2 mg/L respectively, with detailed testing data appended in Table 1. Group 1, 2, and 3 were referring to the students in school 1, 2, and 3.

| Group | Ca (mg/L) | Mg (mg/L) | F (mg/L) | Sr (mg/L) | Ca/Sr | Ca/F | Ca/Mg | Sr/F |
|-------|-----------|-----------|----------|-----------|-------|------|-------|------|
| 1     | 31.8      | 58.1      | 2.80     | 14.2      | 2.24  | 11.36| 0.55  | 5.07 |
| 2     | 90.1      | 44.0      | 1.00     | 3.62      | 24.89 | 90.1 | 2.05  | 3.62 |
| 3     | 47.1      | 10.3      | 0.60     | 0.64      | 73.59 | 78.5 | 4.57  | 1.07 |

Figure 2. Ca, Mg, F, and Sr concentration in drinking water of the surveyed schools.

Table 1. Water quality minerals test results.
5.1.2. Skeletal developments indicators measurement for students. Enrolled students’ height was measured and mean height was calculated. The mean height of each age-group is shown in Table 2. The mean height increased with the increase in age in all three groups. However, the mean height of group 3 aged 11 and 12 was significantly lower than the other two groups \((p<0.05)\), and the height of group 2 and 3 aged 13 was significantly lower than that of group 1, with no difference being detected among students aged 10.

Table 2. Mean height for students at 4 age-groups.

| Age | Group 1 | Group 2 | Group 3 |
|-----|---------|---------|---------|
| 10  | 128.2   | 129.1   | 128.6   |
| 11  | 134.7   | 135.1   | 131.3   |
| 12  | 138.7   | 139.1   | 135.2   |
| 13  | 147.7   | 141.6   | 140.1   |

Bone age of the students, based on their wrist X-ray film, was checked and evaluated. There were 53, 48 and 34 students in group 1, group 2 and group 3 respectively volunteering to take this check. It was observed that 88.24% of students in group 3 having less bone age compared with biological age, which was the highest percentage among the three groups. The difference of bone age and biological age between each two groups was not significant. The details are given in figure 3, below.

Figure 3. Constituent ration of bone age comparing bio age in research groups.

For dental fluorosis, 119, 94 and 133 students in group 1, group 2 and group 3 were diagnosed respectively. In group 1, we found 113 students with dental fluorosis at different degree with 69 students diagnosed as medium or severe degree. In group 2, it was found that 89 students with dental fluorosis at different degree with 58 students diagnosed as medium or severe degree. In group 3, we found 82 students with dental fluorosis at different degree with 24 students diagnosed as medium or severe degree. The DFP was more than 90% in both group 1 and group 2, and saw significant difference with group 3 \((\chi^2=39.80, P<0.01; \chi^2=32.33, P<0.0100)\), which was not different between group 1 and group 2. The DFP and SDFP are shown in figure 4, below.
Figure 4. Prevalence of dental fluorosis.

DCP and ANCT witnessed the most in group 2, and which in group 1 and group 3 decreased successively. DCP was significantly different between each two groups, as group 1 and group 2 ($\chi^2=22.22, P<0.05$), group 2 and group 3 ($\chi^2=45.98, P<0.05$), group 1 and group 3 ($\chi^2=5.70, P<0.05$). ANCT (permanent teeth) witnessed difference between group 1 and group 3 ($t=4.72, P<0.05$), group 2 and group 3 ($t=5.53, P<0.05$). DCP (deciduous teeth) and ANCT (deciduous teeth) of group 2 was the most in the three groups, but there was no significant difference. The details are shown in table 3, below.

**Table 3.** Prevalence of teeth caries and number of caries tooth in the research groups.

| Group | Number of diagnosed students | Deciduous teeth caries (%) | Average number of caries deciduous tooth | Permanent teeth caries (%) | Average number of caries permanent tooth |
|-------|------------------------------|---------------------------|----------------------------------------|---------------------------|----------------------------------------|
| 1     | 116                          | 28.45                     | 0.49                                   | 42.24                     | 0.99                                   |
| 2     | 94                           | 35.11                     | 0.66                                   | 73.40                     | 2.01                                   |
| 3     | 133                          | 29.32                     | 0.59                                   | 27.82                     | 0.53                                   |

5.2. Discussions

5.2.1. Water quality classification. Sr, Ca, Mg and F have all been reported having effects on skeletal development. Ca and Mg are both necessary nutrient, which are important for skeletal and dental development for human [44]. Children demands for Ca and Mg intake could come from various sources besides drinking water. There is no limitation in Chinese drinking water quality standard for Ca and Mg, neither do the other counties so far, only to regulate the upper limit value for hardness considering the taste and scale deposition. Ca is believed to be a major element for bones, and enough Ca intake is vital for children skeletal quality and mineral. Although main Ca intake is from dairy products, Ca in drinking water is still regarded as an important way to obtain as its ionic status [41]. Some researchers put forward that drinking water with lower Ca and higher Mg may have an adverse effect on skeletal development, and suggest the appropriate Ca concentration be 65 mg/L [45].

Mg is regarded as a necessary element for bones and helps to maintain Ca metabolism [46]. Studies have clearly established that F primarily produces effects on skeletal tissues [15]. US New York State
EPA recommends that intake amount of Mg should be within the range of 150～250 mg/d for child and 300～450 mg/d for adult [6]. Assuming drinking water amount is 2 liter per day and 10% of Mg intake comes from drinking water, the agency suggests the Mg concentration should be not exceeding 35 mg/L in drinking water. We use the benchmark of Mg concentration as 45 mg/L to categorize drinking water considering the difference of food nutrient constitution between China and US.

High F exposure will cause fluorosis, while low concentration of F provides protection against dental caries, especially for children [14]. WHO states drinking water with F concentration 0.5～2 mg/L may protect dental caries through incorporation into the matrix of tooth during its formation, development of shallower tooth grooves and tooth surface enamel, and determine the guideline value as 1.5 mg/L [4]. However, Ca has been believed to have antagonistic effect on F with sufficient animal test and intervention trial proof, which may compromise the dental fluorosis at lower or higher F concentration [47-50].

Sr is an important kind of mineral in human bones and teeth and is not currently regulated in drinking water [51]. However, recent studies show that ingestion of Sr may pose a potential threat to human health due to its role in abnormal skeletal developments and bone calcification [52]. EPA listed Sr in the Contaminant Candidate List 3 in 2009. In 2014, EPA announced a preliminary regulatory determination for Sr in drinking water and set the health reference level (HRL) for Sr (II) at 1500 μg/L [6]. However, there is not adequately broad investigation of Sr in drinking water in China. One study in Xi’an city, close to our research location, has reported Sr concentration in drinking water ranged from 0.06 to 1.69 mg/L, with one sample exceeded Health Reference Level of 1500 μg/L. The mean and 95th percentile value of health risk from exposure to Sr was 10−2, within the acceptable limitation of 1 [53]. Based on this result, assuming Sr concentration being exemplified by 10 times, it is still within the health risk acceptable limitation. Therefore, we use 10 mg/L as a classification for Sr concentration in our study.

Categorized the drinking water quality in the three groups, we identified group 1 (high Sr group) as the type of normal Ca, high Mg and high F, group 2 (normal Sr) as the type of high Ca, normal Mg and F, and group 3 (low Sr) as the type of normal Ca, low Mg and F, as shown in table 4.

| Table 4. Concentration of Sr, Ca, Mg and F in the research groups. |
|-------------------------|--|--|--|---|
| Group 1                | ↑ | ↓ | ↑ | ↑ |
| Group 2                | → | ↑ | → | → |
| Group 3                | ↓ | → | ↓ | ↓ |

5.2.2. Effect of drinking water type on skeletal development. According to what we found in literature review, Sr, Ca, Mg and F have effect on skeletal development by all means. However, they compete with each other and show different effect at different intake level.

It was observed that the mean height of group 3 was significantly lower than the other two groups except 10-year-old subgroup. Meanwhile, the percentage of children in group 3 is whose skeletal age being less than age witnessed the most in the three groups. On the other side, children in group 1 did not show being affected by the high concentration of Sr in drinking water as far as body height was concerned. We assume it may be related to sufficient Ca intake and supplement of other nutrients acting synergistic function. Research indicate that Sr was inevitable element for skeleton formation and calcification [54], however, ion exchange happens easily between Sr and Ca which share the similar ionic radius. With Sr deposition amount increasing, bone Ca could be competitively replaced, and calcification of osteogenic tissue repressed [44]. In our study, group 1 with the highest Sr concentration was not observed as undeveloped skeleton compared with the other two groups, even showed better development than group 3 with the lowest Sr concentration. It may imply that drinking water Sr around the 14.2 mg/L may not damage skeletal development.
Group 1 and group 2 did not show obvious difference concerning children’s height and skeletal age even the Ca concentration in group 2 was more than 90 mg/L, much higher than that in group 1. However, Mg and Sr concentration in group 1 were the highest in the three groups as 58.1 mg/L and 14.2 mg/L respectively. We believed Mg and Sr played an important role to help bone calcification, and Sr in drinking water at concentration of 14.2 mg/L did not show any observable adverse effect.

A study suggested drinking water with low Ca (5 mg/L) and high Mg (60 mg/L) have adverse effect on skeletal development based on animal experiment by feeding drinking water of various combined concentration of Ca and Mg [45]. We did not find the similar result in our research. One possibility could be Ca concentration in group 1 was 31.8 mg/L, which was much higher than that extremely low Ca concentration in An’s research. It may imply that Mg with high concentration may not damage skeletal health when there is enough intake of Ca. More research regarded Mg as a major supplementary mineral to prevent and treat osteoporosis through hydroxylation of vitamin D and calcification promotion [40, 46, 55-57].

High concentration of Sr is supposed to lead to skeletal disease under the condition of severe lack of Ca. Animal experiments indicated that Sr have advantageous effect on serum Ca and HOP/Cr, which benefited skeletal development for rats [29]. The result of our study was supportive for the conclusion that Sr at wide range could benefit for skeletal health rather than damage.

5.3. Dental caries and fluorosis.
Lots of research have identified dental fluorosis is positively related to F intake through drinking water [58-60]. The research location we chose was not only with the drinking water Sr, but was an endemic disease region of dental fluorosis, even though drinking water F in group 2 and group 3 did not exceed the national standard as 1.0 mg/L. In our study, DFP in group 1 and group 2 was 94.96% and 94.68% respectively, with no significantly difference between these two groups. DFP in group 3 was significantly lower than the other two groups at 61.65%. Actually, we did not expect such a high DFP in group 2, as its water F concentration did not exceed the national standard. We suspected it may be caused by the existence of Sr, which may accelerate replacing of Ca by F. It indicated that Sr could be the risk factor for children’s dental health when F intake is at the similar level. An animal experiment had the similar result as ours. This experiment fed rats in 5 groups with water at the same concentration of F and different concentration of Sr, and found the rats being fed with water free from Sr was the latest to be found spots in their tooth enamel. Furthermore, the rats drinking water with higher concentration of Sr were identified as more severe tooth enamel spots. It was believed that Sr could replace Ca ion in the form of Ca hydroxide phosphate and stimulate reaction between F and hydroxyl [23].

Some researchers reported that Mg could be a protective factor for dental fluorosis [61-62]. We did not find similar evidence in our study as the highest exposure group to drinking water Mg did not seem to have better outcome of dental fluorosis. Dental fluorosis happening may be resulted from the joint exposure of high F and Sr, but Mg intake from drinking water was not insufficient enough to take effect. In this regard, we were not able to conclude since we did not evaluate Mg intake amount from food.

We also diagnosed dental caries for the children and found the group 2 had the highest DCP besides the most DFP. Moreover, the group 2’s DCP (permanent teeth) was significantly higher than the other two groups. In this regard, we could not see F and Sr in drinking water at concentration of 1.00mg/L and 3.62 mg/L acting protectively on dental caries as the previous studies indicated. Research reported that Sr in drinking water was negatively related to dental caries happening [54, 63], and Sr in caries was significantly lower than that in health tooth, which may indicate Sr intake benefitting prevention of dental caries [4, 29, 35, 64]. But we did not find the result that children with higher Sr intake developing less dental caries.

On the other side, drinking water F concentration standard varies worldwide for its effect on tooth. It was stated that 0.5~2.0 mg/L F in drinking water would protect children from tooth caries, but other research found 0.9~2.5 mg/L F in drinking water may cause dental fluorosis and dental caries...
[65-69]. As F exposure also could be from food and inhalation in some regions, it makes more difficult to determine a widely criteria for F in drinking water [70]. F tolerance dose was affected by intake of Ca, Mg and protein, and people with higher Ca, Mg and protein intake were less sensitive to F [47, 71-74].

6. Conclusion
This study investigated Ca, Mg, F, Sr in drinking water and height, bone age, dental caries and dental fluorosis of students aged from 10 to 13, as indicators of their skeleton development and health in Pucheng county in Shaanxi province, Northwest China. Since concentration regulation for Sr, Ca and Mg in drinking water is rare, we refer to some literature and local standard to determine the concentration level for each researched ion, except F. According to this, the drinking water of three studied student groups was classified as high Sr, low Ca, high Mg and high F for group 1, normal Sr, high Ca, normal Mg and F for group 2, and low Sr, normal Ca, low Mg and F for group 3.

The students in high Sr, low Ca, high Mg and high F were regarded as the most well-developed as far as height was concerned, except students aged at 10. And the percentage of students being diagnosed as bone age lagging behind bio age was found the most in group 3 with low Sr, normal Ca, low Ca and low F drinking water. The percentage of students identified as dental fluorosis and permanent dental caries was both found the most in group 2 with normal Sr, high Ca, normal Mg and F drinking water.

This study indicated that Mg and Sr played an important role to help bone calcification, and Sr in drinking water at concentration of 14.2 mg/L did not show any observable adverse effect. However, when there is co-existence of Sr and F in drinking water, we suggest a stricter limit of F, for Sr may accelerate replacing of Ca by F and cause dental fluorosis.

7. Recommendations
Since the dose-response relationship and health outcome of many minerals in drinking water have not been determined, especially when there is complicated synergistic or antagonistic effect, these minerals have not been included in drinking water guideline. Despite of the difficulties, some research have got progress in animal experiment, more population observation and long-term cohort studies are needed in this area for better understanding and help for drinking water quality guideline amendment.

8. Ethics
The research protocol has been reviews by ethics review committee in National Center for Rural Supply Technical Guidance, Chinese Center for Disease Control and Prevention and was approved. We contacted the school’s deans and shared our purposes and what activities we would conduct, and got their agreement before we determined the researching schools. Prior to any substantial question was asked, or physical data was collected, we provided consent letter for every student aged 10-13, all of 4th-6th grade and partly from 3rd grade as our potential researching objectives, and made sure they understand it was totally voluntary to be included in.

9. Limitations
A large part of mineral intake comes from food, like Ca and Mg rather than drinking water, and dental caries prevalence can be affected by toothbrushing habit and daily hygienic behavior. Although we selected the schools located in neighboring villages with similar dietary habit and income, due to the research fund limitation, we did not investigate children’s diet and nutrients intake amount, and how many times they brushed their teeth or what kind of toothpaste they used, some with F added. All these factors may have impact on the result and analysis to some extent. Meanwhile, we did not investigate household drinking water quality, which would impact the intake amount of all kinds of minerals through drinking water.
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