Analysis and health risk assessments of some trace metals in Ethiopian rice (white and red) and imported rice

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ABSTRACT

In this study, the levels of some trace metals (Cr, Cu, Mn, Pb, and Zn) in Ethiopian and imported rice samples were analysed. The rice samples were digested with a mixture of HNO3:HClO4:H2O2 (3:2:1 v/v) at a temperature of 200 °C for 2:30 h. The mean concentrations (mg/kg) of metals in Ethiopian and imported rice samples were found in the range of 4.82–17.04 for Cr, 11.30–18.30 for Cu, 6.04–9.22 for Mn; and 17.15–27.37 for Zn, within FAO/WHO limits. However, Pb was not detected in both rice samples. The Red rice contained higher levels of metals compared with the White rice. The Ethiopian rice recorded higher levels of metals than the imported ones. The estimated daily intake (mg/kg-day) was found to be at a safe level with reference to the maximum tolerable daily intake. Except for Cr, the values of the target hazard quotient (THQ) were also within the safe level in all the samples, posing no potential health risks on consuming rice. The hazard index values (HI) for the metals in Ethiopian rice except Jimma Red were slightly higher than unity, indicating the alert threshold level and potential health risks to rice consumers. Thus, the concentrations of these metals were less than the maximum limits set by FAO/WHO limits and most of THQ and HI values less than unity. Therefore, there was no serious noncarcinogenic risk to human health from exposure to metals through the consumption of these rice.

1. Introduction

Rice (Oryza sativa L.) is among the major cereal crop consumed as a staple food by over half of the world’s population [1,2], particularly in Asian countries such as China, Thailand, Japan, and Indonesia, which are the world’s leading rice producers [3,4]. China is the largest producer and consumer of rice, accounting for over 30% of worldwide rice output [5,6,7]. It is most widely farmed, consumed and the most nutritious grains. Rice is a rich source of starch and contains a small amount of protein, fat, fibres, minerals and vitamins [8,9].

Oryza sativa L. is the most often farmed rice species in Asia, while Oryza glaberrima S. is the most commonly cultivated rice species in Africa, with only minor physical differences. Other members of the Oryza genus are not generally farmed, but in times of food scarcity, humans used to harvest some indigenous species [10,11]. Rice is the most well-known source of food for low-income countries in Africa, playing a critical role in poverty alleviation [12]. According to reports, the number of rice growers increased from 53 thousand in 2006 to around 284 thousand in 2009 [3].

Agriculture is one of the pillar of Ethiopian economy. Although rice production has a short history in Ethiopia, it is the potential crop for production and considered as strategic food security crop. Rice production has brought significant impact in the lives of large populations and created employment opportunities for many citizens in different parts of the country [13,14].

Essential metals play a key role in metabolic pathways. However, heavy metals such as As, Cr and Pb are toxic, not easily biodegradable and biologically accumulate for long time in the environment [15]. These metals are toxic even at low concentration and can cause severe health effects such as renal, cardiovascular, central nervous system disorder, skin irritations, nausea, bone diseases, gastrointestinal problems and a variety of cancers [4,12,16]. Cereal crops like rice may be contaminated with toxic metals from soil, water, pesticide application, chemical fertilizers, industrial activities and transportation [4].

Certain heavy metals are essential for plant growth at low levels, but some metals are highly toxic to humans. These toxic heavy metals can be absorbed, accumulated by plants and eventually enter the human body through food intake [17]. Cu, for instance, may cause irritation of nose,
eyes and mouth; and cause headaches, stomach aches, dizziness and vomiting [18]. Pb contamination also adversely disrupts the mental growth, neurological and cardiovascular systems in humans. Besides, Pb can cause adverse kidney malfunction and high blood pressure in adults [19].

Excess levels of Zn in the human body can disrupt the immune system and change the concentration of high-density lipoproteins. Because of its significance in metabolic processes as well as the functioning of the neurological, immune, and reproductive systems, Mn bioavailability should also be highlighted [15].

In various countries the mineral contents of rices were reported [20, 21, 22, 23]. To the best of our knowledge few researches have been done to investigate the concentration of trace metals in rice samples in both imported and Ethiopian rice [11, 24].

Therefore, the objective of this study was to compare the levels of trace metals (Cr, Cu, Mn, Pb, and Zn) in Ethiopian rice (White and Red) to commercially available imported rice from India, Pakistan, and the Republic of Korea; and to evaluate the non-carcinogenic health risks of trace metals in rice samples.

2. Materials and methods

2.1. Description of the study area

The study areas in Ethiopia were Fogera, Pawe, and Jimma. Fogera is located in the South Gondar Zone, Amhara Regional State. The district is located at 11°46' N latitude and 37°33' E longitude with an elevation of 1100 m above sea level. Jimma is located in the southwestern highlands of the Oromia Regional State, which is found at 7°40' N latitude and 36°20' E longitude at an elevation of 1100 m above sea level. Jimma is located in the Benshangul Gumuz Regional State and is situated at 11°19' N latitude and 36°19' E longitude at an elevation of 1100 m above sea level. These districts were selected as study sites based on predominant potential areas of rice production. However, the imported White rice samples from India, Pakistan, and the Republic of Korea were purchased in the marketplaces of Gondar Town, Ethiopia.

2.2. Instrumentation and chemicals

The instruments used for analysis of rice samples were digital analytical weighing balance (Adam AAA 100 LE), refrigerator (Hitachi, LR902T, England), Kjeldahl apparatus (Gallenkamp, England) and Flame Atomic Absorption Spectrophotometer (BUCK SCIENTIFIC MODEL 210 VGP, USA), a sieve (0.5 mm and 2 mm, ASTM E11 UK), Whatman filter paper (No. 42) and an oven (GallenKamp, UK).

Chemicals of analytical grade (Anala R) and deionized water were used throughout the experiment. Digestion was made using HNO₃ (69–72% Spectrosol, BDH, England), HClO₄ (70% Qualkins), and H₂O₂ (30%, Okhla industrial area, New Delhi, India). The standard stock solutions (1000 mg/L) of Cr, Cu, Mn, Pb, and Zn were used to prepare intermediate and calibration standards for constructing calibration curves for analysis of metals in both non-spiked and spiked experiments.

2.3. Sampling and sample pretreatment

Fogera rice samples were collected from Fogera Agricultural Research Institute (FARI), Woreta, while samples from Jimma and Pawe were collected directly from the farmers. About 3 kg White and Red rice samples per each location, namely Fogera, Jimma, and Pawe samples were collected. Besides, 1.0 kg imported rice samples belonging to the countries (India, Pakistan and Republic of Korea), were collected from the local market in Gondar Town, Ethiopia. The collected samples were then separately packed into clean polyethylene bags, labeled and brought to the laboratory for further analysis.

Both the local (White and Red) and imported rice samples were thoroughly washed with tap water followed by deionized water and then dried to a constant weight until they were crisp and brittle. The dried rice samples were then ground using a plastic pestle and mortar, and sieved through a 0.5 mm nylon in order to remove large particles, ready for further digestion [11].

To optimize the digestion procedure, 0.5 g of each rice sample was digested with a mixture of 3 mL of HNO₃ (69–72%), 2 mL of HClO₄ (70%) and 1 mL of H₂O₂ (30%) at 200 °C for 2:30 h in a dry and clean 100 mL borosilicate digestion flask on a Kjeldahl apparatus until clear and colorless solutions were obtained. Among the digestion procedures, the one which required minimum volume of acid ratios at a minimum digestion time and temperature were considered as optimum conditions for the analysis. The digested solutions were cooled for 10 min and deionized water was added to every sample and filtered with Whatman (No. 42) filter paper into a 50 mL volumetric flask followed by dilution with deionized water up to the mark. The blank was also digested under similar digestion procedures in parallel with the sample. Finally, the digested and diluted sample solutions were kept in the refrigerator at a temperature of 4 °C until analysis [25].

2.4. Method validation

The operating parameters such as analytical wavelengths, slit width, lamp current, energy and oxidant/fuel type were presented in Table 1.

A 10 mg/L of intermediate solutions of each metal were prepared from 1000 mg/L standard solutions to establish the calibration curves. The concentrations of the metals were determined based on the regression equation described in Table 2. Similarly, the levels of metals in the blank solution were determined following the same analytical procedure as the sample.

The regression coefficients (R²) of the calibration were greater than 0.99, indicating that there was a very good association between absorbance and the concentration of metals. The detection limits were calculated as the concentrations that provide signals equal to three times the standard deviations of the blanks [26]. The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample and quantitatively calculated with acceptable uncertainty using 10 times the standard deviation of the blank. The results indicated that the limit of detection (LOD) for Cr, Cu, Mn, Pb and Zn were 0.080, 0.051, 0.023, 0.002 and 0.008 mg/L, respectively (Table 2). The LOQ values were ranged from 0.020 to 0.27 mg/L.

The validity (accuracy) of the analytical procedures was tested by spiking known concentrations of standards into the rice samples [7, 27] and it was presented as percent recovery. The percent recovery values of Cr, Cu, Mn and Zn were 94.70%, 99.10%, 101.9% and 95.8%, respectively.

2.5. Health risk assessment

The health risks of trace metals from long-term rice consumption by humans [6, 28, 29] can be evaluated in terms of their carcinogenic and non-carcinogenic effects [30]. The target hazard quotient (THQ) and hazard index (HI) were used to describe the possible health risks to humans [31, 32].

Estimated daily intake (EDI) was used to express non-carcinogenic health risk of metals to humans, which was calculated by Eq. (1).

### Table 1. Operating parameters for FAAS.

| Metals | Wavelength (nm) | Slit width (nm) | Lamp current (mA) | Energy (J) | Flame type |
|--------|----------------|----------------|-------------------|------------|------------|
| Cr     | 357.9          | 0.7            | 2                 | 3.845      | Air-C₂H₂   |
| Cu     | 324.0          | 0.7            | 1.5               | 3.798      | Air-C₂H₂   |
| Mn     | 279.5          | 0.7            | 3                 | 4.093      | Air-C₂H₂   |
| Pb     | 283.2          | 0.7            | 2                 | 3.695      | Air-C₂H₂   |
| Zn     | 213.9          | 0.7            | 2                 | 3.13       | Air-C₂H₂   |

FAAS = Flame Atomic Absorption Spectrophotometer.
EDI = \frac{CmRI}{BW} \quad (1)

where, Cm denotes metal concentration, RI is rice consumption rate per day (gram/day person), and BW is body weight. An average body weight of 65 kg was taken for adults in the current study areas of Ethiopia. The rate of consumption of rice per day could influence the tolerance of metal contaminants. Rice was thought to be the smallest amount often consumed food in Ethiopia, with an average daily intake of 15 g was supported based on the National Food Consumption Survey [33].

Target hazard quotient (THQ) was used to estimate the non-carcinogenic risk to humans from long term exposure to trace metals from rice consumption and was expressed in Eq. (2):

\[ THQ = \frac{EDI}{RI} \quad (2) \]

where, RfD stands for reference dose, which is expressed in mg/kg/day. The RfD values reported in mg/kg/day were 0.003 for Cr; 0.04 for Cu, 0.14 for Mn and 0.3 for Zn [34]. If THQ ≥ 1, there may be a concern about potential human health risks caused by exposure to non-carcinogenic elements, whereas, if THQ < 1, there is no concern about potential human health risks caused by exposure to non-carcinogenic elements [35].

Hazard index (HI) indicates the total target hazard quotient (THQ), which reflects the non-carcinogenic danger posed by individual trace metals and is calculated according to Eq. (3):

\[ HI = \sum_{i=1}^{n} THQ_i \quad (3) \]

If the value of HI ≤ 1, it is assumed that non-carcinogenic risks have no substantial impact. If HI > 1, however, there is a risk that non-carcinogenic effects will occur, and the likelihood increases as HI increases [26,36].

2.6. Statistical analysis

Analysis of data was performed using the latest SPSS version 23.0 package. The concentration and the health risk of metals (Cr, Cu, Mn, Pb, Zn) were assessed by the SPSS version 23.0 package. One way ANOVA was used to compare whether the difference between the mean concentration of metals in all rice samples was significant or not [37]. Pearson’s correlation analysis was employed to identify the relationship between metal concentrations. All the statistical tests were conducted at a 95% confidence level.

3. Results and discussion

3.1. Levels of metals in rice samples

The levels of metals are listed in Table 3. Other elements, except Pb, were detected in all rice samples. The Cr levels in all the Ethiopian rice samples were ranged from 8.84-17.04 mg/kg (the highest in Fogera Red and the lowest in Jimma White), which were not significantly different except for Jimma White. However, Cr concentrations in imported rice were ranged from 4.82 to 11.02 mg/kg and differed significantly from Ethiopian rice. The levels of Cr in rice samples from India (4.82 mg/kg) were significantly different from Pakistan (5.56 mg/kg) and the Republic of Korea (11.02 mg/kg). The levels of Cr in the current study were in a good agreement with the results reported from Nigeria [12] and Tanzania [38], but their levels were higher than reported from Ethiopia [11] and Jordan [39].

The levels of Cu in Ethiopian rice samples followed the increasing order of Jimma White < Pawe White < Pawe Red < Foger Red < Jimma Red < Fogera Red. There were no significant variations in the levels of Cu in all Ethiopian rice samples. Similarly, in the imported rice, Cu was found in the order of India < Pakistan < Republic of Korea, with no significant differences in their levels (p < 0.05). The levels of Cu were similar to the results reported from Tanzania [38]. However, it was higher than previously reported from China [22], Ethiopia [11], Jordan [39], India [40] and Nigeria [12].

The levels of Mn in Ethiopian rice samples were found between 6.04 mg/kg and 8.05 mg/kg. The Pawe Red rice had the highest mean Mn levels, whereas the Pawe White rice had the lowest. In imported rice samples, the lowest and the highest levels of Mn were detected in India and the Republic of Korea, respectively. There were significant differences in Mn concentrations among the three sites via Fogera, Jimma and Pawe (P < 0.05). In the imported rice, it was observed that the levels of Mn were significantly different. The levels of Mn in Ethiopian rice samples were in good agreement with that reported from Ethiopia [11], Nigeria [12], Jordan [39] and India [40]. Though the level of Pb has been reported previously in rice [11,12,38,40], in this study, it was found below the detection limit.

Zinc is required by plants in largest amount and the contents in the rice samples were in the range of 22.01 mg/kg (Pawe White) to 27.37 mg/kg (Jimma White). The amount of Zn did not show any significant variation between Fogera White, Jimma White and Pakistan; Foger Red, Pawe Red and Pawe White; and between India, Pakistan and Republic of Korea rice samples. In contrast, the lowest and highest levels of Zn were detected in rice samples imported from India and Pakistan, respectively. The levels of Zn in the present study were to some extent comparable

| Rice sample source | Trace Metals concentrations (mg/kg) |
|--------------------|-----------------------------------|
|                    | Cr  | Cu  | Mn   | Pb   | Zn   |
| Fogera White       | 16.10 ± 2.04  | 12.70 ± 0.60  | 6.34 ± 0.20  | ND  | 27.37 ± 3.00  |
| Fogera Red         | 17.04 ± 1.53  | 13.60 ± 1.02  | 6.44 ± 0.30  | ND  | 26.67 ± 1.50  |
| Jimma White        | 8.84 ± 0.506  | 11.30 ± 1.00  | 8.00 ± 0.04  | ND  | 27.00 ± 3.01  |
| Jimma Red          | 13.30 ± 0.891  | 12.74 ± 0.40  | 7.89 ± 0.01  | ND  | 25.00 ± 4.00  |
| Pawe White         | 15.10 ± 1.802  | 11.63 ± 1.801 | 6.04 ± 0.046 | ND  | 22.01 ± 2.015 |
| Pawe Red           | 15.40 ± 1.205  | 11.87 ± 0.874 | 8.05 ± 0.017 | ND  | 23.02 ± 2.020 |
| India              | 4.82 ± 0.023   | 16.68 ± 1.351 | 8.00 ± 0.752 | ND  | 17.15 ± 0.003 |
| Pakistan           | 5.56 ± 0.0155  | 17.28 ± 0.006 | 7.32 ± 0.020 | ND  | 25.23 ± 0.003 |
| Republic of Korea  | 11.02 ± 0.201  | 18.30 ± 0.013 | 9.22 ± 0.001 | ND  | 19.05 ± 0.002 |
| WHO/FAO Safe limit | 20  | 40   | 500  | 5.0 | 60 |

ND = not detected, *WHO/FAO Source (FAO/WHO, 2001). The values in the same column followed by different letters are significantly different (p < 0.05).

Each rice sample was analyzed by triplicate measurements.
with results reported by Tegegne et al. from Ethiopia [11] and India [40]. The levels of Zn were also found within the range of results reported from Jordan [39]. However, the levels of Zn in the present study were higher than results reported from Nigeria [12] and China [22], but lower than reported from Tanzania [38].

As shown in Table 3, from Ethiopian rice samples, Zn was found to be the highest in concentration followed by Cr except in Jimma White rice, but Mn was the lowest in concentration in Ethiopian rices. Similarly, the imported rice contained the highest amount of Zn followed by Cu. The levels of all trace metals analyzed in rice samples, in this study, were lower than the permissible limits set by WHO/FAO [41]. This confirms that the daily intake of rice under this study did not pose any risk.

The observed differences in the mean concentrations of metals between imported and Ethiopian rice samples might mainly be accounted for the differences in soil types, agricultural inputs, and species differences [11]. In general, the highest metal content occurs in Ethiopian rice samples than in rice imported from India, Pakistan, and the Republic of Korea.

Pearson's correlation was applied to determine the relationships between different metal contents in the rice samples and the results are presented in Table 4. Strong positive correlations were observed between the concentrations of Cu with Mn in Fogera Red (0.829), Jimma Red (0.922) and Jimma White (0.948), Cu with Zn in Fogera White (0.970), Jimma White (0.8), Pawe Red (0.662) and Pakistan (0.938), Mn with Zn in Pawe red (0.683), Pawe White (0.997), India (0.891) and Jimma White (0.632); Cr with Cu in the Republic of Korea (0.778) and Cr with Mn in Jimma White (0.632), which suggests the origin of metals were from similar sources [42]. Strong negative correlations were observed between Cr and Cu in Fogera Red (–0.722) and Fogera White (–0.756); Cr and Mn in Jimma Red (–0.654), Pawe Red (–0.993), Pawe white (–0.902) and India (–0.946); Cr and Zn in Fogera white (–0.614), Pawe Red (–0.761), Pawe white (–0.931), India (–0.898) and the Republic of Korea (–0.998); Cu and Zn in Republic of Korea (–0.778). This indicates the lack of common origin between metals [43].

### 3.2. Health risk assessment

As shown in Table 5, the EDI values of Cr, Cu and Zn (mg/kg-day) were found to be comparable in all the study sites (ranging from 0.0029–0.0040 for Fogera Red; 0.0031–0.0062 for Fogera White; 0.0020–0.0063 for Jimma Red; 0.0029–0.0058 for Jimma White; 0.0027–0.0051 for Pawe Red and 0.0027–0.0053 for Pawe White). It could also be noted that comparable EDIs of Mn were observed in Fogera Red (0.0115 mg/kg-day), Fogera White (0.0016 mg/kg-day) and Pawe Red (0.0014 mg/kg-day) rice, while virtually similar EDIs of Mn were recorded in Jimma Red (0.0018 mg/kg-day), Jimma White (0.0017 mg/kg-day) and Pawe White (0.0018 mg/kg-day) rice. The EDI values of the investigated metals were found in the order of Zn > Cu > Mn. This suggested that the EDI values (mg/kg) of metals were found to be at safe level compared with the maximum tolerable daily intake (MTDI) of Cr (0.2), Cu (3.0), Mn (5.0) and Zn (60.0) [44,45].

The values of THQ are given in Table 6. The results showed that THQ values were ranged from 0.3708 to 1.317 for Cr; 0.0651 to 0.1056 for Cu; 0.0999 to 0.0152 for Mn; and 0.0134 to 0.0208 for Zn giving the general order of Cr > Cu > Zn > Mn for target adults in all samples. It should be noted that the THQ values, for all the trace metals except Cr, were less than unity, indicating that metal intake would have no significant health risks in the study areas. However, Cr could cause health risks to human except Jimma Red and imported rices.

The hazard index (HI) values of trace metals through consumption of rice by adults, in this study, are presented in Table 6. The HI values of metals in both domestic and imported rice samples followed the order: India < Pakistan < Jimma Red < Republic of Korea < Jimma White < Pawe Red < Pawe White < Fogera Red < Fogera White. The HI values of the metals in rice samples from India (0.4933), Pakistan (0.5587), Jimma Red (0.7792) and the Republic of Korea (0.9815) were less than unity, suggesting that the consumption of rice does not pose any potential health risk. However, the HI values of rice from Jimma White (1.1290) < Pawe Red (1.2554), Pawe White (1.2840), Fogera Red (1.3355), Fogera White (1.4204) were greater than unity, indicating that there is health risks associated with metals due to consumption of rice. Chromium was the higher contributor for HI, which was consistent with the results reported by Meseret et al. [46,47]. It might surpass the level of concern (HI < 1) for Cr and Cu in Fogera Red (1.2554), Pawe Red (1.2840), Fogera Red (1.3355), Fogera White (1.4204) were greater than unity, indicating that there is health risks associated with metals due to consumption of rice. Chromium was the higher contributor for HI, which was consistent with the results reported by Meseret et al. [46,47]. It might surpass the level of concern (HI < 1)
Table 6. Hazardous quotient (THQ) and hazard index (HI) values of Cr, Cu, Mn, Pb, Zn metals in adults via the consumption of rice.

| Rice Type         | Cr  | Cu   | Mn   | Zn   |
|-------------------|-----|------|------|------|
|                   | THQ | THQ  | THQ  | HI   |
| Fogera Red        | 1.2384 | 0.0732 | 0.0104 | 0.0134 | 1.3355 |
| Fogera White      | 1.3107 | 0.0784 | 0.0106 | 0.0205 | 1.4204 |
| Jimma Red         | 0.6800 | 0.0651 | 0.0132 | 0.0208 | 0.7792 |
| Jimma White       | 1.0230 | 0.0735 | 0.0131 | 0.0192 | 1.1290 |
| Fawre Red         | 1.1615 | 0.0670 | 0.0099 | 0.0169 | 1.2554 |
| Fawre White       | 1.1846 | 0.0685 | 0.0132 | 0.0177 | 1.2840 |
| India             | 0.3708 | 0.0962 | 0.0133 | 0.01308 | 0.4933 |
| Pakistan          | 0.4277 | 0.0997 | 0.0121 | 0.0192 | 0.5587 |
| Republic of Korea | 0.8462 | 0.1056 | 0.0152 | 0.0146 | 0.9815 |

> 10) for long term exposure [49]. An agreement between THQ and HI would play paramount role in the provision of completed risk assessments associated with the ingestion of metals via rice grown in these areas. However, the EDI, THQ and HI values of metals in imported rice were less than unity, which suggests that there were no adverse health effects on consumption of imported rice samples.

It is observed that the THQ and HI values higher than 1, was mainly contributed by the chromium. This might partly be attributed to higher concentration of chromium in the rice plant emanated from applications of excess agricultural chemicals like fertilizers to increase yields/production of rice. Moreover, pesticides and herbicides are also applied to control the pests and weeds [48,49]. It should also be noted that the extreme lower value of Rfd as a denominator might also be account for the higher values of HI and THQ [50].

4. Conclusion

The level of metals (Cr, Cu, Mn, Zn, and Pb) in two Ethiopian rice varieties and three imported commercially available rice samples were determined. All the samples contained the highest amount of Zn. A comparison between the mean concentrations of metals in Ethiopia and the imported rice from India, Pakistan, and the Republic of Korea showed significant differences for most of the metals at 95% confidence levels. All the detected metals in both Ethiopian and imported rice samples were below the limits set by FAO/WHO. Analysis of the health risk of trace metals due to consumption of rice grown in Ethiopia was conducted. The values of THQ and HI exceeded unity with the higher contribution by Cr, bearing a high risk of trace metal to adult consumers due to long term exposure from consumption of local rice. The study marked a clear picture of biota contamination through the proper channels of consumers due to the transferability of the trace metals. As a result, further health risk assessments of trace metals due to consumption of other cereal crops should be conducted as there is very limited relevant data in the local areas of interest.

Declarations

Author contribution statement

Atanfu Guadie, Ibrahim Mohammed: Conceived and designed the experiments; Performed the experiments.
Tamene Beshaw: Analyzed and interpreted the data; Wrote the paper.
Molla Tefera: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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The authors are unable or have chosen not to specify which data has been used.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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