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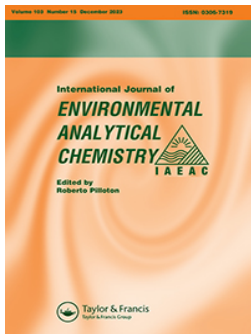


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Health and ecological risks of selected heavy metals in sugarcane (*Saccharum Officinarum* L.) and its soil of plantation in Sanka irrigation farmland, Northeast, Ethiopia

Tigabu Awwaris Dejen and Asamene Embiale Taye

Department of Chemistry, College of Natural and Computational Science, Woldia University, Woldia, Ethiopia

ABSTRACT

The ecological and health risks posed by heavy metals (HMs) have been a major concern of the world. This study aimed to assess the potential health and ecological risks of Fe, Pb, Cr, Zn, Co, Ni, and Cd metals in the sugarcane and its growing soil at Sanka farmland, Northeast Ethiopia. The sampling was conducted at six locations in three potential sugarcane cultivation Kebeles. HMs were quantified using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). They were found in the range of 2297 (Fe) to 3.750 (Co) mg/kg for soil and 0.557 (Fe) to 0.020 (Zn) mg/L for sugarcane. The metal transfer factors of the investigated HMs were 0.0002 (Fe) to 0.384 (Co), indicating a low sugarcane response to HMs. Six pollution indices (three multi-metal ecological impact indicators and three single-metal ecological effect indicators) were applied to the data. All multi-metal ecological impact indicators show that the soil is severely polluted. In contrast, the single-metal ecological effect indicators showed severe pollution by Fe and Cd, moderate pollution by Pb and Cr, and low pollution levels by Ni, Co and Zn. Apart from Cd, Cr and Pb in the soil sample and Zn and Cd in the sugarcane juice sample, all HMs concentrations were below allowable limits set by World Health Organization (WHO). The contribution of each exposure route to the total intake of HMs showed the trend of non-dietary oral intake > dermal contact > juice drinking > inhalation route. Single-metal health risk assessment (hazard quotient) revealed no significant non-carcinogenic risk for adults and children, while multi-metal health risk assessment (hazard index) indicated children have high non-cancer risks. Similarly, the single-metal cancer risk assessment showed no cancer risk, except Cr, whereas the multi-metal cancer risk assessment showed children and adults inhabitants likely to have cancer in their lifetime.

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1. Introduction

Health hazards include physical, chemical and biological factors affecting a person's health and the environment, which concern the globe. Among the chemical hazardous substances, HMs are the major concern of the globe due to their serious health

CONTACT Asamene Embiale Taye ✉ asamene@wldu.edu.et

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implication for all living things in general and humans in particular. The HMs were mainly derived from anthropogenic sources (such as mining processing and smelting, biomass burning, chemical production, vehicles, agricultural imputes, factory waste and wastewater irrigation) and natural (volcanic eruptions, forest fires, sea-salt sprays, biogenic activities, rock weathering and wind-borne soil particulate matters) sources [1–3]. Although some HMs such as Zn, Fe and Cr (III) are essential for metabolic processes and may show nutrient deficiency when relatively low availability, other HMs such as Cd, Ni, Pb and Hg are poisonous even in trace amounts [4]. HMs can have cumulative negative effects that induce chronic health impacts such as degenerative disorders, particularly in the brain system, kidney and liver failure, ataxia, depression, lymphocytosis, and microcytic hypochromic anaemia. They can also be teratogenic and carcinogenic in some situations through the mechanism of enzymatic inhibition, poor antioxidant metabolism, and oxidative stress may all play a role. Many of the adverse health effects caused by heavy metals are caused by the creation of free radicals, which cause DNA damage, lipid peroxidation, and a reduction of protein sulfhydryls (e.g. glutathione) [5,6].

Humans are exposed to HMs by inhalation, oral intake (either dietary or non-dietary), and dermal contact absorption [7]. Among the exposure routes, oral intake/ingestion through consumption of foods contaminated with heavy metals contributes up to 90% of exposure. Thus, the constant consumption of foods to get essential nutrients tend to increase levels of HMs in the food chain [8,9]. The bioaccumulation of HMs varies across different plant species. Some can absorb and retain specific HMs more than others by using their leaf, root, stem, or fruit parts exposed to contaminated water, air, and soil [10,11].

Consequently, the HMs contamination of food by HMs is a vital environmental problem and becoming increasingly severe worldwide, particularly in developing nations [12]. The great concern of HMs in developing countries is because of the fast and unorganised industrialisation, the use of a large quantity of pesticides, herbicides, organic and inorganic fertilisers in agricultural sectors, and poor management of municipals waste (in big cities and small towns) in developing nations [10,13,14]. The most prominent source of food contamination is the cultivation of plants in polluted soil; the plants grown in such soil could absorb and retain high amounts of HMs [10,15].

Sugarcane is associated with the *Graminaceae* family, and its genus is *Saccharin*, which originates in tropical South and Southeast Asia and then expands to other parts of the world [16]. The sugarcane plant is one of the top-ranking crops worldwide and is cultivated in more than 110 countries with a production rate of 1869.7 million tons per year [17]. However, it has high accumulated HMs beyond those permitted by the World Health Organization (WHO) or United State Environment Protection Agency (USEPA) that prolonged consumption of contaminated sugarcane could harm human health [18,19]. In Ethiopia, two types of sugarcanes (*Tafach Shenkora* and *Key Shenkora* in Amharic) that belong to the *Saccharum spontaneum* species were found. These types of sugarcane plants have been cultivated in different parts of Ethiopia; one of the large cultivation areas of sugarcane is located in Northeast Ethiopia, at the Sanka farmland [20,21]. Farmers in the study area have utilised massive amounts of organic and inorganic fertilisers, insecticides, pesticides, and herbicides to increase the production yield of sugarcane and other cereal crops, which might increase the level of HMs in the farmed soil. In addition, the topography of the farmland is found at the foot of the surrounding

mountains, which eroded soil from the top of mountains has a high probability of increasing the level of HMs in the growing soil of the sugarcane plant.

Moreover, the farmland is located near the main highway that connects the Afar and Amhara regional states, where an enormous number of heavy vehicles are going daily, increasing the soil pollution by HMs. Consequently, direct exposure to the soil or the chronic consumption of crops such as sugarcane plants growing on this farmland may cause a variety of health problems to inhabitants besides the impact on the ecosystem as a whole. Nevertheless, as far as our survey goes, no study has yet to be conducted to determine HMs in sugarcane and its plantation soil in Ethiopia in general and Sanka farmland in particular. Therefore, it is necessary to quantify the current level of HMs, compare it with the standard values, and estimate the future impact on the ecosystem and inhabitants of this farmland.

This study aimed to investigate (1) the level of Pb, Fe, Zn, Cr, Ni, Co, Cd in sugarcane plants and their underlying soil at Sanka irrigation farmland in North Wollo, Ethiopia, (2) the HMs associated with carcinogenic and non-carcinogenic (toxicological) risks of adult and children inhabitants (using USEPA protocol) through soil (non-dietary) exposures and consumption of sugarcane juice, (3) the ecological risks (using the parameters such as ecological risk index (ERI), contamination factor (CF), geo-accumulation index (I_{geo}), contamination degree (C_d), metal transfer factor (MTF), pollution index (PI), and risk index (RI)) due to exposure of contaminated soil and consumption of sugarcane juice were also estimated.

2. Materials and methods

2.1. Description of the study area

The research was carried out at Sanka irrigation farms in the Amhara region, North Wollo Zone, Gubalafto Woreda. Sanka is a village located 23 kilometres west of the capital city of the North Wollo Zone (Woldia), at an elevation of 2077, and an exact location between 11,053' 40.325"N and 39,027' 24.852"E. The average household size was 4.15 people. The majority of the population (86.6%) practiced Ethiopian Orthodox Christianity, while 13.32% identified as Muslim. Almost everyone belonged to the Amhara ethnic groupings and spoke Amharic as their primary language. The average annual lowest and maximum temperatures are 7°C and 27°C, respectively, with annual minimum and maximum rainfall of 1.2 mm and 414.8 mm, with relative humidity ranging from 40 to 74% [22]. The sampling sites for the study is illustrated in [Figure 1](#).

2.2. Sampling procedures

The sampling of soil and sugarcane samples was performed based on both purposive and simple random sampling techniques. Thus, a preliminary reconnaissance survey was conducted to identify possible sampling sites in the research area's cropland. After surveying, three potential sugarcane production Kebeles namely Geshober, Anova, and Woynye, were selected as sampling sites. In addition, the sampling Kebeles were chosen based on geography and irrigated water source. Following the selection of Kebeles, six sampling points were established (two for each Kebele) from three sampling Kebeles.

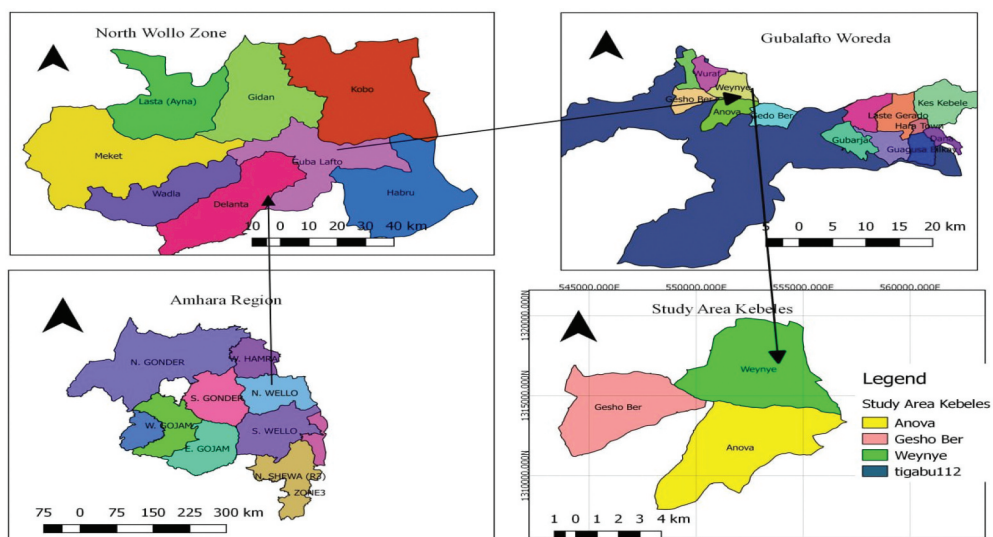


Figure 1. The geographical location of Sanka farmland, Ethiopia.

Then, six sugarcane plants weighing about 0.5 kg each were gathered using a stainless-steel knife. The shoots were removed, and the stems were split into smaller pieces before being tied by rope and brought to the laboratory. Finally, to eliminate dirt, the sugarcane was rinsed with ultrapure water (18.2 MΩ.cm). The sugar cane juice (SJ) was then extracted into a 50-mL flask using a pre-cleaned extractor and chilled for further digestion examination [23].

A 3 kg composite soil sample was collected into clean plastic bags from the exact locations as the sugarcane samples (500 g from each of the six locations). A steel auger was used to collect soil samples from 0–20 cm depth, which were then pooled together to generate a composite sample. The soil samples were collected and then transported to the laboratory; it was air-dried for two weeks in a dry and dust-free environment at room temperature (25°C); then an oven dry was performed until constant weights were achieved. The materials had been homogenised after being crushed with a mortar and pestle and passed through a 2 mm sieve. Finally, the homogenised, dried, and sieved soil samples were placed in polyethene bags and placed in desiccators until digestion and analysis could take place [14,23].

2.3. Digestion of sugarcane juice (SJ) and soil samples

The digestion of sugarcane juice was performed using a method established by [14]. To make it brief, 20 mL of the SJ sample was transferred to a 250-mL round-bottom flask, and 6 mL of aqua Regia (3:1 ratio of HNO₃ and HCl) was added. Then the solution was digested in the microwave for 3:00 h at a temperature of 210°C. After 3:00 h of digestion, the digested solution was permitted to cool to room temperature for about 30 min without disassembling the condenser. The solution was then diluted with 10 mL of ultrapure water while the condenser apparatus was being disassembled. After disassembling the equipment, 10 mL of ultrapure water was added to the solution.

This was accomplished by cleaning the neck of the round bottom flask and the tip of the condenser that was in contact with the residue created during cooling and minimising dilution of filter paper by digesting residue while filtering with Whatman 110 mm filter paper into a 50 mL volumetric flask. Finally, the cooled solution was filled to the mark with ultrapure water.

On the other hand, the soil sample was digested as about 1.25 g of the composite dried, sieved, and homogenised soil sample was digested with 20 mL of aqua regia in a beaker (open beaker digestion) on a thermostatically controlled hot plate. The solution mixture, was heated to near dryness and cooled to room temperature. Then 5 mL of H₂O₂ was added in parts to complete the digestion and the resulting mixture was heated again to near dryness in a fume cupboard. The walls of the beaker were cleaned with 10 mL of ultrapure water, followed by 5 mL of HCl, and then heated again. The resulting digest solution was allowed to cool and put into 50 mL of the standard flask through Whatman 110 mm filter paper and filled up to mark with ultrapure water, which was ready for analysis of Fe, Pb, Cr, Zn, Co, Ni, and Cd heavy metals [24].

The digestion procedure was done three times for the soil as well as for sugarcane samples. The digestion of the blank reagent was also carried out by following similar procedures simultaneously with the sample. All the solutions were stored in the refrigerator until HMs were analysed.

2.4. Heavy metals analysis of sugarcane juice and soil samples

After the SJ and soil sample were digested, the level of Fe, Pb, Cr, Zn, Co, Ni, and Cd HMs were quantified by using an inductively coupled plasma – optical emission spectrometry (ICP-OES) with the detection range (mg/L) were Fe (0.0018–7.2), Pb (0.001–1.68), Cr (0.008–1.68), Zn (0.002–3.6), Co (0.007–1.68), Ni (0.009–3.07), and Cd (0.013–1.68). For each target analyte, the device was calibrated using a series of standard solutions. Thus, calibration metal standard solutions for each HMs in soil and SJ sample were made from an intermediate standard solution containing 100 mg/L from standard stock solutions with 1000 mg/L. Each working standard for each metal of interest was obtained using ultrapure water. For each sample, three replicate determinations were performed.

The measurement validation in this study was checked by the instrument's linearity, accuracy, precision, and detection limit (DL). Thus, the linearity was performed by the calibration curve's coefficient of correlation (R^2) from one set of experimental measurements. Whereas the accuracy and precision of the method were achieved using standard deviation (SD) and present recovery test respect for each target analyte [14,25,26]. The percentage recovery of the analyte was calculated by using formula 1:

$$\% \text{Recovery} = \frac{\text{Amount after spike} - \text{Amount before spike}}{\text{Amount added}} \times 100 \quad (1)$$

The instrumental detection limit, method detection limit and limit of quantification were also assessed. The manufacturer of the ICP_OES instrument provided the instrument detection limit, while the method detection limit (LOD) and limit of quantification (LOQ) can be calculated by multiplying the standard deviation of the blank (SD_b) by three times ($LOD = 3SD_b$) and multiplying the standard deviation of the blank (SD_b) by ten times ($LOQ = 10SD_b$) respectively the results were given in supplementary Table S3 [27].

2.5. Pollution indices

In the current study, different indicators have been used to compare the amount of HMs, and background value in irrigated soil to describe the contamination entering the cultivation's ground. Multi-metals and single-metal indices parameters, including ecological risk index (ERI), contamination factor (CF), geo-accumulation index (I_{geo}), contamination degree (C_d), metal transfer factor (MTF), pollution index (PI), and risk index (RI) have been employed. The formulas used for each parameter are given in Equations (2)–(8).

2.5.1. Single-metal pollution index parameters

2.5.1.1. Contamination factor (CF). The contamination factor parameter can describe a given hazard substance contamination and is calculated as Equation (2) [28].

$$CF = \frac{C_i}{C_0} \quad (2)$$

where (C_i) is the soil HMs concentration, HMs level of the background value (C_0), which is world average value of soil was used for this study [29], CF is the ratio of the metal concentration. The level of metal contamination was defined as low ($CF < 1$), moderate ($1 < CF < 3$), significant ($3 < CF < 6$) and very high contamination ($CF > 6$) [30].

2.5.1.2. Metal transfer factor. The metal transfer factor is used to suggest the bioavailability of each HMs in the soil that is affected by the combinations of environmental, biological and chemical parameters [31]. MTF also used to know the transmission of HMs from soil to sugarcane plant. It is calculated as a ratio of the concentration of a specific HM in the plant tissue (mg/kg dry weight (D_w)), such as a leaf, stem, root, fruit, etc., to metal concentration in soil (mg/kg D_w). If MTF is greater or equal to unity ($MTF \geq 1$), the absorption of HMs from the soil by the sugarcane is higher. A lower MTF value, on the other hand, indicates that the plant has a poor response to metal absorption. Thus, the sugarcane plant was used for the calculation of MTF in this study by utilising the formula 3 [26,32]:

$$MTF = \frac{C_{plant}}{C_{soil}} \quad (3)$$

Where; C_{soil} and C_{plant} are the concentration of metals in the soil and the concentration of metals in the plant respectively.

2.5.1.3. Geo-accumulation index (I_{geo}). I_{geo} is used to evaluate the pollution level of metal contamination. It can be calculated using the formula 4:

$$I_{geo} = \log_2(C_n / 1.5B_n) \quad (4)$$

C_n is the observed element content in soil (mg/kg), and B_n is world average background level of heavy metal (HM) (mg/kg) is used to investigate minor anthropogenic effects. Factor 1.5 is applied to limit the effect of any variations in the background or control values that may be related to lithogenic variations in the sediment. $I_{geo} > 5$ indicates that the soil is highly contaminated; $3 < I_{geo} \leq 4$ revealed that the soil is heavily contaminated; $2 < I_{geo} \leq 3$ confirmed that the soil is moderate to heavily contaminated; $1 < I_{geo} \leq 2$

showed that the soil is moderately contaminated; $0 < I_{\text{geo}} \leq 1$ revealed that the soil is uncontaminated to moderately contaminated [30].

2.5.1.4. Ecological risk index (ERI). The ERI quantified the probable ecological risk of several factors. The ERI is determined by using Equation (5):

$$ERI = Tr \times PI \quad (5)$$

where Tr indicates a toxic-response factor, each element has its own Tr values, which are given as a standard (i.e. Mn = 10, Ni = 5, Cu = 5, Zn = 1, Cd = 30, Hg = 40, Pb = 5, As = 10, and Cr = 5) [26,33–35]. The pollution degree of the soil on the bases of ERI values is classified as $ERI < 40$ is low HMs pollution; $40 \leq ERI < 80$ is moderate HMs pollution; $80 \leq ERI < 160$ is higher HMs pollution; $160 \leq ERI < 320$ is serious HMs pollution; $ERI \geq 320$ is severe HMs pollution [36,37].

2.5.2. Multi-elemental pollution indices parameters

The commutative pollution effect of HMs were estimated using C_d , PI and RI parameters. The equation from 6 to 8 have been used to find the value of each parameters. Hence, C_d , is defined as the sum of all individual HM contamination factors, whereas PI is used to describe the comprehensive pollution level based on the mean and maximum values of each heavy metals [28,38]

$$C_d = \sum_{i=1}^n CF^i \quad (6)$$

$$PI = \sqrt{\frac{(CF_{\text{ave}})^2 + (CF_{\text{max}})^2}{2}} \quad (7)$$

CF^i , CF_{ave} , and CF_{max} , represent the CF for an individual element, the mean of CFs, and the maximum CF, respectively. C_d is the degree of contamination for multiple elements. The degree of soil pollution can be categorised as unpolluted soil ($C_d < 1.5$), slightly polluted soil ($1.5 \leq C_d < 2$), moderately contaminated soil ($2 \leq C_d < 4$), moderately heavily polluted soil ($4 \leq C_d < 8$), severely polluted soil ($8 \leq C_d < 16$), heavily contaminated soil ($16 \leq C_d < 32$) and extremely contaminated soil ($C_d > 32$), whereas PI can be used to classified the soil pollution as unpolluted soil ($PI < 0.7$), slightly contaminated soil ($0.7 \leq PI < 1$), moderately polluted soil ($1 \leq PI < 2$), severely polluted soil ($2 \leq PI < 3$) and heavily contaminated soil ($PI > 3$) [30]

2.5.2.1. Risk index (RI). The RI used for estimated the commutative effect of HMs and it was determined according to Equation (8). The degree of contamination can be classified into 4 categories based on the RI values $RI < 150$ showed low HMs contamination, $150 \leq RI < 300$ showed moderate HMs contamination; $300 \leq RI < 600$ showed high HMs contamination, and $RI \leq 600$ showed very high HMs contamination [36,37].

$$RI = \sum_1^n ERI \quad (8)$$

Where RI is used to quantitatively express the potential risk of all measured HMs by summing the ecological risk factors of the individual HMs [28].

2.6. Health risk assessments

The health risk assessment is an approach to estimate the type and magnitude of past, present, and future health risks in individuals, groups of individuals, and communities. The cancer and non-cancer health risks for adults and children due to HMs intake by soil dust and SJ were assessed based on the established method of USEPA. Generally, the two prominent parameters called target hazard quotient (THQ) and target hazard index (THI) have been employed for non-cancer risk estimation while cancer risk (CR) and total cancer risk (TCR) were employed for cancer risk estimation [39,40].

2.6.1. HMs intake dose assessment

The chronic average daily doses (ADD) and estimated chronic average daily intake (EDI) of HMs from agricultural soil and SJ have been assessed, respectively. Residents could obtain HMs from the soil via many methods, including oral consumption (non-dietary), skin contact, and breathing. On the other hand, HMs from sugarcane plants can enter into body via oral consumption of SJ. The estimated HMs intake from soil (expressed as the chronic average daily dose, ADD) was assessed using the formulas listed as Equations (9)–(11) while EDI was assessed using Equation (12) [41–44].

$$ADD_{oral} = \frac{C_s \times IngR \times EF \times ED}{BW \times AT} \times 10^{-6} \quad (9)$$

$$ADD_{inh} = \frac{C_s \times InhR \times EF \times ED}{BW \times AT \times PEF} \quad (10)$$

$$ADD_{derm} = \frac{C_s \times SA \times AF \times ABS \times EF \times ED}{BW \times AT} \times 10^{-6} \quad (11)$$

$$EDI = \frac{C_j \times FIR \times EF \times ED}{BW \times AT} \times 10^{-3} \quad (12)$$

Where; ADD_{oral} , ADD_{inh} , and ADD_{derm} are chronic average daily intake from the soil in ($\text{mg kg}^{-1} \text{ day}^{-1}$) by oral intake (unintentional exposure by non-dietary mouth intake of dust or air-borne soil particles), inhalation, and dermal contact of soil dust respectively; C_s is the HMs content in the soil; EDI is the estimated chronic average daily dose intake through sugarcane juice (mg/kg day^{-1}); C_j is HMs concentrations squeezed sugarcane juice (mg/kg). Since there is no data available for FIR (food intake rate) of sugarcane for Ethiopian adults and children, this study has used the mean of 325 g/person/day for adult and 150 g/person/day for children were used in calculating the EDI values derived from the average daily consumption of vegetables suggested by WHO guidelines [45]. The average weight of adult and children person were considered to be 60.7 and 15 kg, respectively which is site-specific, and the definition, values, and units of ED, IngR, PEF, SL, InhR, AT, EF, BW, ABS, AF, and SA, parameters, including in Equation (9)–(12) are given in Table S1 [46].

2.6.2. Non-cancer risk assessment

Assessing non-carcinogenic human health risks from non-dietary soil intake and oral intake of SJ were estimated using target hazard quotient (THQ) and target hazard index

(THI) parameters. THQ is used to estimate the non-cancer risk of single metal non-cancer risk. Thus, the THQ_s (target hazard quotient for soil sample) values of the local population due to the non-dietary intake of soil dust in three pathways (THQ_{inh} , THQ_{derm} and THQ_{oral}) were calculated using Equations (13)–(15) while THQ_j (target hazard quotient for SJ sample) values are calculated based on Equation (16) [47].

$$THQ_{inh} = \frac{ADD_{inh}}{RfD_{inh}} \quad (13)$$

$$THQ_{oral} = \frac{ADD_{oral}}{RfD_{oral}} \quad (14)$$

$$THQ_{derm} = \frac{ADD_{derm}}{RfD_{derm}} \quad (15)$$

$$THQ_j = \frac{EDI}{RfD_{oral}} \quad (16)$$

$$THI_s = \sum THQ_s \quad (17)$$

$$THI_j = \sum THQ_j \quad (18)$$

Where; EDI is the estimated chronic average daily dose of HMs by SJ intake, ADD_{inh} , ADD_{oral} and ADD_{derm} are the chronic average daily dose of HMs by the population from soil dust (mg/day/kg) through inhalation, oral intake and dermal contact respectively and THQ_{inh} , THQ_{derm} and THQ_{oral} represent total hazard quotient for inhalation, total hazard quotient for dermal and total hazard quotient for oral route. The RfD is the reference dose (mg/kg/day) for each of the HMs of interest (supplementary Table S2).

On the other hand, the total hazard index of soil (THI_s) and the target hazard index of SJ (THI_j) were employed to investigate the health risks induced through multiple HMs. THI_s calculations have been done by considering four scenarios. Thus, (1) a single metal dose across three routes, (2) multiple metals in a single exposure route, (3) multiple elements in any of the three exposure routes and (4) multiple elements in four routes (soil dust in three routes and SJ in oral route). The THI_j has been calculated based on the scenario of multiple elements in the oral route only. The general formula for calculating THI_s and THI_j is given in Equations (17) and (18), respectively. According to USEPA guidelines, if the THIs or THI_j , or THQ value became less than unity, there is no apparent health impact due to the metals considered. However, the THIs or THI_j , or THQ value is greater than unity, indicating potential health impact implications. A severe chronic health impact has been suggested for THIs or THI_j , or THQ greater than 10 [23,47].

2.6.3. Carcinogenic risk assessment

The possible incremental cancer risk (CR) posed due to the intake of single carcinogenic HMs and cumulative cancer risk (TCR) due to the intake of more than one carcinogenic HMs for soil and SJ were estimated using Equations (19)–(22) [48,49].

$$CR_s = ADD \times SF \quad (19)$$

$$TCR_s = \sum_{n=1}^i CR_s \quad (20)$$

$$CR_j = EDI \times SF \quad (21)$$

$$TCR_j = \sum_{n=1}^i CR_j \quad (22)$$

where $i = 1, 2, 3, \dots, n$, CR_s = cancer risk for soil sample; CR_j = cancer risk for sugarcane juice; TCR_s = total cancer risk for soil sample; TCR_j = total cancer risk for juice sample; SF = slope factor for carcinogenicity; EDI = estimated chronic average daily intake of HMs; ADD = chronic average daily dose of heavy metals in different soil exposure routes in mg/kg/day [23]. The SF is a slope factor value of the evaluated HMs and is given in Tables S2 [44,50,51]. Where TCR or CR is classified as very low ($<10^{-6}$), low ($10^{-5} < TCR < 10^{-6}$), medium ($<10^{-4}$), high ($<10^{-3}$) and very high ($>10^{-3}$) [47]

2.7. Data analysis

All data in this study were subjected to Pearson Correlation analysis to correlate the levels of heavy metals in sugarcane or soil sample. The amounts of HMs in sugarcane and soil samples were given as arithmetic mean $\pm$ standard deviation (SD). SPSS software version 26 has been used for calculating the mean and Pearson Correlation analysis. Analysis of variance (ANOVA) test was performed to see the significant variation of HMs in soil and sugarcane samples. A 95% confidence level has been used for all significant tests in this study [52].

3. Results and discussion

3.1. Validation of the method

The method used in this study was validated by performing calibration of ICP-OES, calculating percent recovery and blank analysis. Thus, the calibration curve exhibited good linearity, with R^2 values ranging between 0.9957 and 0.9999, which were greater than the acceptable limit of 0.9995 for the linearity. The concentration of the working standards, correlation coefficient, and calibration equation of the calibration curve for each metal are shown in supplementary Table S3 [53].

The percent recoveries for the studied metals have been made using spiking of standard in the soil and sugarcane samples, and the percent recovery for soil and sugarcane juice was found between 81.98 and 105%, and 89.4 and 105.2%, respectively. All the recovery values were within the acceptable range of 80–120%. In addition, the precision of the method was also calculated and expressed as the RSD of the three replicated readings. The RSD values obtained for sugarcane juice and soil samples ranged from 0.02–5.00% and 0.60–3.78%, respectively, which was under the required control limits of 0–15% [53]. The details of the result are given in Table S4.

The Limit of Quantification (LOQ) and Method Detection Limits (MDL) for all metals addressed in this study were derived using a standard formula from the response of seven replicates of the reagent blank, $LOQ = 3 SD$ and $MDL = 10SD$, respectively and the Method MDL that laid for sugarcane juice and soil samples were in the range of 0.003–0.252 mg/L,

and 0.009–0.015 mg/kg respectively while the LOQ values for the sugarcane juice and soil sample were found between 0.01 and 0.84 mg/L and 0.09–21 mg/kg respectively. Furthermore, the instrument detection limit (IDL) obtained from the instrument's operation manual ranges from 0.001 to 0.009 mg/kg. The details of the result for the MDL, LOQ, and IDL were given in supplementary Table S5.

3.2. Concentration of heavy metals in a soil sample

The concentration HMs in the soil sample was found in the range of 3.75 (Co) to 22,975 (Fe) mg/kg with the increasing order of $\text{Co} < \text{Ni} < \text{Cd} < \text{Pb} < \text{Zn} < \text{Cr} < \text{Fe}$. Apart from Cd, Cr, and Pb in a soil sample, all HMs concentrations are below FAO/WHO allowable limits for agricultural land [54,55]. Table 1 contains information regarding the arithmetic mean concentrations of HMs in the soil sample.

The concentration of the investigated metals in soil samples was compared to previously reported data at national and international levels. Thus, the level of Fe found in this study is greater than the sample taken from Dukem, Ethiopia and Nigeria [54,55]. The Cd level in this investigation was lower than in soil samples taken in Dukem, Ethiopia. Still, its amount is higher than in soil samples from east Gojam, Ethiopia [56,57]. The mean concentration of Pb in this study is lower than the reported value in farmland in China and Shanghai agricultural soil [43,44]. However, the Pb levels in the current study were greater than in the soil of Southern Ethiopia [42,58]. Similarly, the chromium (Cr) concentration in the present work was much greater than those discovered in soil samples from East Gojam, Ethiopia and Pakistan [26,44]. Furthermore, the current study's Co, Ni, and Zn level were lower than those reported in soil samples in Iraq, Brazil, and Indian farmland, respectively. Co and Ni concentrations were also lower than reported in East Gojjam Zone, Ethiopia [22,42–44,50,56,59].

In general, the distribution of heavy metals in soil across a region or within a given country may be due to the various soil types in the area, the use of different farming techniques, as well as the geographic and climatic diversity of the nations, the application

Table 1. The mean, minimum and maximum concentration of HMs in soil and SJ sample along with WHO/FAO standards.

	HMs in soil sample (mg/kg)				HMs in sugarcane juice sample (mg/L)			
	Mean $\pm$ SD	Min	Max	WHO/FAO	Mean $\pm$ SD	Min	Max	WHO/FAO
Fe	22975 $\pm$ 220	22740	23178	50,000	0.557 $\pm$ 0.008	0.549	0.564	0.010
Pb	101 $\pm$ 3.820	100.400	101	100	0.033 $\pm$ 0.001	0.176	0.204	0.010
Cr	129 $\pm$ 4.160	127.200	131	100	0.127 $\pm$ 0.002	0.124	0.130	0.100
Zn	121 $\pm$ 3.940	120.858	121	300	0.020 $\pm$ 0.001	0.010	0.030	5.000
Co	3.750 $\pm$ 0.087	3.650	3.80	50.0	0.036 $\pm$ 0.002	0.034	0.038	0.001
Ni	7.07 $\pm$ 0.104	6.950	7.15	50.0	0.026 $\pm$ 0.004	0.023	0.031	0.002
Cd	14.740 $\pm$ 0.089	6.950	7.13	3.00	0.022 $\pm$ 0.004	0.020	0.020	0.100

Table 2. The pollution indices value for I_{geo} , CF, ERI and MTF parameters.

	Fe	Ni	Co	Zn	Cd	Pb	Cr
I_{geo}	4.94	−1.93	−0.971	0.38	3.16	1.43	1.03
CF	45.95	0.39	0.77	1.95	13.4	4.04	3.07
ERI	−	1.96	−	1.95	402	2.2	15.36
MTF	0.000024	0.00368	0.0096	0.00017	0.0015	0.00033	0.00098

of some organic amendments, such as compost made from sewage sludge or sewage sludge, the excessive use of various pesticides, fertilisers, and herbicides, and the release of municipal and industrial pollutants [22,42–44,50,54–59].

3.3. Heavy metals concentration in sugarcane juice

The mean level of the sugarcane juice sample showed a wide range, with values from 0.02 (Zn) to 0.557 (Fe) mg/L with the order of $Zn < Cd < Ni < Pb < Co < Cr < Fe$. The level of Cr and Fe was relatively higher than other investigated metals. This variation might be because chromium contaminations primarily emanate from fertilisers, organic manure, and anthropogenic activities. Fe has large crustal abundances, which could contribute more to the current study area. Apart from Zn and Cd in SJ sample, all HM concentrations were determined to be above the acceptable allowed limits specified for food by the Food and Agriculture Organization (WHO) and World Health Organization (FAO) [19]. The details about the mean concentrations of HMs in the SJ are given in Table 1. The HMs level in SJ in the current study was lower than in some previous reports. For instance, the average concentration of Fe in Nigeria and Kenya, Cr in India, Zn in China and Kenya, Co in India and Nigeria, Cd in India, China, and Japan, and Ni in India were higher than the current study [19,26,58,60,61]. As the same time, the level of Pb in the present study was higher than that of the previous studies from India [60] and Nigeria [48]. This concentration variation might attributed to soil pH, organic matter type and distribution, plant age, micro- and macronutrient availability, and plant genotypes vary from region to region [62].

3.4. ANOVA and correlation analysis

The Pearson correlation coefficient (r) measures the strength of the linear link between two variables and attempts to build a line of best fit through the data of the two variables [53]. Thus, the correlation values are categorised as no correlation ($r = 0.00–0.19$), low correlation ($r = 0.20–0.39$), medium correlation ($r = 0.40–0.59$), higher correlation ($r = 0.60–0.79$) and highest correlation ($r = 0.8–1.00$) [53]. The Pearson correlations tests for investigating HMs in the sugarcane juice and soil samples are summarised in supplementary Table S6. Thus, the highest, medium, and low positive correlations in sugarcane samples were seen in Cr/Fe, Ni/Pb, Cr/Cd and Cd/Fe; Cr/Pb, Co/Zn, and Ni/Cr; Pb/Fe, Ni/Fe, and Cd/Zn respectively. Similarly, the correlation coefficients between metals in soil were investigated. The highest correlation was seen between Co/Cr, Co/Zn, Cd/Cr, Cd/Zn, and Cd/Ni, while the higher positive correlation was observed between Co/Pb, Cd/Pd, and Cd/Co. A medium-positive correlation is also observed between Zn/Pb, Zn/Cr, Ni/Co, Ni/Fe, and Ni/Pb. The correlation coefficients between the metals Pb/Fe and Cr/Fe in the soil sample have a low positive correlation. The correlation coefficients between metals Fe/Zn and Cu/Pb in the soil have no correlation coefficient. Generally, the highest and higher positive correlation revealed that the paired HMs might have similar sources or are probably controlled by similar processes. In contrast, a low positive correlation indicates that paired metals have different origins or sources [22]. Moreover, the ANOVA test of HMs showed a significant difference within each sample and between the soil and sugarcane juice with p value of less than 0.05.

3.5. Pollution indices

The degree of soil pollution was assessed using I_{geo} , CF, ERI, MTF, C_d , RI and PI parameters. The single-metal pollution indices can be estimated using I_{geo} , CF, ERI and MTF parameters and the result of investigated HMs were found in the range of -1.93 (Ni) to 4.94 (Fe), 0.39 (Ni) to 45.95 (Fe), 1.95 (Zn) to 402 (Cd) and 0.000024 (Fe) to 0.0096 (Co), respectively while the multi-elemental effect were assessed using C_d , RI and PI parameters, with the value of 9.34 , 441 and 32.4 respectively. The trends of HMs for I_{geo} and CF parameters were $Ni < Co < Zn < Cr < Pb < Cd < Fe$, whereas their trend for MTF and ERI were $Fe < Zn < Pb < Cr < Cd < Ni < Co$ and $Zn < Ni < Cr < Pb < Cd$, respectively.

Generally, the finding of this study showed that RI, C_d , and PI values indicate that the soil is highly polluted. Nevertheless, the I_{geo} , ERI, and CF values revealed that Fe and Cd metals exhibit severe pollution, Pb and Cr exhibit moderate pollution, and Ni, Co, and Zn exhibit low pollution levels. Moreover, the MTF value of all target heavy metals was less than one, indicating the sugarcane plant's poor response towards heavy metal absorption and the sugarcane plant. Moreover, although the MTF value for all HMs is below one, Co metal has the highest MTF value, which indicates that the sugarcane plant has a relatively high response to the Co metal [16,22]. The variation of MTF for each metal might be due to the difference in mobility in a natural occurrence in soil, the selective absorptivity nature of the plant and retention in the soil [31]. The details of the results for each parameter are presented in Table 2.

3.6. Health risk assessments

The most common techniques that can be used for estimating the health risk (cancer and non-cancer) due to HMs exposure are calculating HQ, CR, TCR and HI. However, four basic scenarios should be considered while calculating the HQ, CR, TCR and HI parameters. Thus, a single metal can enter the body by either a single route or multiple routes, which can have different impacts. On the other hand, multiple metals can also enter the body through single or multiple routes, which can have various health impacts. Such scenarios can be used to know the synergetic effect of multiple routes on a single metal or multiple metals in a single exposure route. Moreover, it is also advantageous to identify the primary exposure route and prominent metals that can cause high impact, which helps to take remedial action at a particular point. Therefore, four risk scenarios employed in this study are: 1) the risk of a single metal entering each exposure route, 2) the risk of a single-metal entering all exposure routes, 3) the risk of multi-metals that enter through each route, and 4) risk of multi-metals that enter through all of the exposure routes. The details values for both cancer risk and non-cancer risk estimator parameters are given in Table 3.

3.6.1. Exposure doses

The chronic average daily intake dose of HMs by different routes of exposure for adults and children was calculated using Equations (9)–(12). The average time required for calculating cancer and non-cancer risks is different, resulting in an enormous difference in intake value. Hence, the calculated chronic daily doses for adults and children are presented in supplementary Table S7 after the references. The chronic daily intake of HMs from soil dust for adults through inhalation, dermal contact and ingestion routes were

HQ and HI for non-cancer risk assessment

CR and TCR values for cancer risk assessment

Cancer risk for Adult

£ indicate over all cancer risk values for all HMs by any of exposure routes for soil and juice samples, * indicates total cancer risk values of each metal in all exposure routes for both soil and juice sample, § indicates total cancer risk values of all HMs in each exposure routes for both soil and juice sample, ** indicates total non-cancer risk values of each metal in all exposure routes for both soil and juice sample, § indicates total non-cancer risk values of all HMs in each exposure routes for both soil and juice sample and £ indicate over all non-cancer risk values for all HMs by any of exposure routes for soil and juice samples

found in the range of $2.9\text{E}-10$ (Co) to $3.6\text{E}-06$ (Fe), $1.2\text{E}-07$ (Co) to $1.5\text{E}-03$ (Fe) and $3.1\text{E}-06$ (Co) to $3.79\text{E}-02$ (Fe) respectively while that of children were $1.1\text{E}-10$ (Co) to $8.6\text{E}-04$ (Fe), $3.8\text{E}-06$ (Co) to $3.06\text{E}-01$ (Fe) and $1.4\text{E}-09$ (Co) to $6.5\text{E}-05$ (Fe) respectively. Thus, the result showed chronic daily intake through dermal contact > ingestion > inhalation for adults and ingestion > dermal contact > inhalation for children. Furthermore, the highest and the lowest chronic average daily dose of HMs for children and adults were found in non-cancer risk ADD and cancer risk ADD values, respectively, except for dermal exposure routes, in which the reverse is true. The EDI values for children and adults through the consumption of SJ were found in the range of $1.5\text{E}-06$ (Zn) to $5.6\text{E}-04$ (Fe) and $5.4\text{E}-06$ (Zn) to $3.0\text{E}-03$ (Fe), respectively. EDI values used to estimate cancer risk are relatively higher than EDI values used for estimating non-cancer risk. Generally, the chronic average daily intake of HMs from the soil sample was higher than that obtained from sugarcane juice for children and adults. Thus, wearing of face mask or minimising unnecessary time spent on farmland might decrease HMs exposure.

3.6.2. Characterization of non-carcinogenic health risks

The non-cancer and cancer risks of HMs via three non-dietary and one dietary exposure routes for children and adult inhabitants were assessed. The results obtained showed that dermal contact > non-dietary oral intake > oral dietary intake of SJ > inhalation for adults and non-dietary oral intake > dermal contact > oral dietary intake of SJ > inhalation for children. Iron (Fe) had the highest non-carcinogenic risk in children and adults for all three non-dietary routes, whereas Co was the lowest. For other HMs, the order of non-carcinogenic hazards (high to low) in oral intake, halation, dermal contact, and SJ consumption for adults and children was $\text{Co} < \text{Ni} < \text{Zn} < \text{Pb} < \text{Cr}$; $\text{Ni} < \text{Zn} < \text{Cr} < \text{Pb} < \text{Cd} < \text{Fe}$; $\text{Ni} < \text{Zn} < \text{Cr} < \text{Pb} < \text{Cd} < \text{Fe}$ and $\text{Zn} < \text{Fe} < \text{Ni} < \text{Pb} < \text{Co} < \text{Cr} < \text{Cd}$ (Table 3). Although the THQ values for all heavy metals are below the acceptability level (1.0), Fe, Pb, and Cd contribute significantly in both children and adults. These values showed a potential non-carcinogenic health risk from ingesting SJ and soil dust was considered safe [47]. Moreover, the data also showed that the risk levels of non-dietary risk (soil dust) for all target heavy metals are relatively lower than those of their corresponding sugar juice-drinking risk.

The sum of risks induced by multiple elements was assessed using THI and TTHQ by considering three scenarios 1) total non-cancer risk of each metal intake by four exposure routes (oral intake of SJ (TTHQ) and soil intake by inhalation (HI_{inh}), ingestion (HI_{ing}) and dermal contact (HI_{derm})), 2) total non-cancer risk of all HMs intake by each of four exposure routes, 3) overall total non-cancer risk of all HMs intake by four of exposure routes. Thus, the HI values of Fe, Zn, Pb, Cr, Cd, Ni and Co intake by any of the exposure routes for adults were found in the range of 0.179 (Cr) to 0.0008 (Zn), with the order of $\text{Zn} < \text{Co} < \text{Ni} < \text{Pb} < \text{Fe} < \text{Cd} < \text{Cr}$, for that of children it was 0.491 (Cd) to 0.0018 (Co) with the increasing order of $\text{Co} < \text{Zn} < \text{Ni} < \text{Pb} < \text{Cr} < \text{Fe} < \text{Cd}$. The HI value of all HMs across each exposure route for adults was found in the range of $6.03\text{E}-05$ (HI_{inh}) to 0.279 (HI_{derm}) following the order of $\text{THI}_{\text{inh}} < \text{TTHQ} < \text{THI}_{\text{ing}} < \text{THI}_{\text{derm}}$ while that of children were $1.5\text{E}-04$ (THI_{inh}) to 0.964 (THI_{ing}) with the order of $\text{THI}_{\text{inh}} < \text{TTHQ} < \text{THI}_{\text{derm}} < \text{THI}_{\text{ing}}$. This result revealed children were more suspected of non-cancer risk than adults, although the hazard index is less than unity. Moreover, the overall total hazard index of all heavy metals due to intake by any of the four exposure routes for adults and children were 0.428 and 1.177, respectively,

which indicates children's THI is greater than the threshold values set by USEPA that they might have high non-cancer risks [63].

3.6.3. Characterization of carcinogenic health risks

The single-metal and multi-metals cancer risks of children and adults in this study were conducted based on four scenarios: 1) single metal risk in each route, 2) single metal risk in all routes, 3) multi-metals risk in a single route, and 4) multi-metals risk in all routes. Hence, the multi-metal cancer risk of adults and children across each exposure route was found in the order of $CR_{oral} > CR_{derm} > CR_{inh} > TCR_j$, while the multi-metal cancer risk of adults and children by all exposure routes were 0.005 and 0.0056, respectively. The cancer risk of adults and children for every single metal in all exposure routes was found in the range of $5.16E-06$ (Ni) to $5.0E-03$ (Cr), with the order of $Ni < Pb < Cd < Cr$ and $6.03E-06$ (Ni) to $5.5E-03$ (Cr), with similar order of adults. The single metals risk of adults and children through inhalation, dermal contact, oral intake of soil dust, and drinking of SJ were obtained in the ranking of $Ni < Pb < Cd < Cr$; $Cd < Pb < Ni < Cr$; $Ni < Pb < Cd < Cr$ and $Cd < Pb < Ni < Cr$ respectively.

Generally, cancer and non-carcinogenic health risk assessment results show that drinking HMs-contaminated SJ poses lower risks than the overall non-dietary exposures for the investigated HMs. Moreover, among the investigated metals, Cr risk values for adults and children were above the guideline set by USEPA (10^{-4}) [47]. Although the study showed children are more susceptible than adults, adults and children living in the study area are likely to develop cancer in their lifetime. Nevertheless, all other HM's risk values are below the USEPA standard, except Cd, which is in a tolerable range.

4. Conclusion and recommendation

This study assessed the level of HMs in soil and sugarcane plants and their ecological and health risk in Sanka farmland in northeast Ethiopia. The Pb, Cr, and Cd level in soil and Fe, Pb, Cr, Ni, and Co in sugarcane juice were above the maximum tolerable levels set by FAO/WHO. The Pearson correlation result indicated that Fe, Cr, and Cd as well as Ni and Pb might have come from similar sources. ANOVA test confirmed that the level of HMs in the soil and SJ significantly differ ($p < 0.01$). Dermal contact and non-dietary oral intake are ranked top for the chronic average daily intake of HMs by adults and children, respectively. The HQ for Cr and Cd indicates they were predominately cause for the non-carcinogenic risk of adults and children. The overall hazard index revealed that children are at potential non-cancer risk. Moreover, the single-metal cancer risk values for adults and children were below the allowable limit set by USEPA (10^{-4}), except Cr, which was above the guideline. The multi-metal cancer risk (TCR) values were above the USEPA guidelines, except for inhalation and dermal routes for soil and oral intake of SJ. Generally, both cancer and non-carcinogenic health risk assessments due to the drinking of SJ poses lower risks than the overall non-dietary (soil dust) exposures of HMs.

Since the ecological and health risk assessment showed that HMs could severely impact the ecosystem and humans, prolonged studies including investigating soil pH, salinity, alkalinity, electrical conductivity, and the nature of agricultural inputs like pesticides and herbicides, were highly recommended. Identifications of the main sources of HMs and remedial action should be taken. It also suggested that farmers minimise synthetic

fertilisers, pesticides, and herbicides or use alternatives such as organic compost. Furthermore, researching different types of agricultural products commonly cultivated in the study area and other pollutants, including chlorinated pesticides and herbicides, is highly recommended.

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Ethical responsibilities of authors

All authors have read, understood, and have complied as applicable with the statement on 'Ethical responsibilities of Authors' as found in the Instructions for Authors.

Data availability statement

The data used to support the findings of this study are included in this study.

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