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UPTAKE OF TOXIC TRACE METALS (CD, PB)  
AND MICRO NUTRIENTS (CU, ZN, MN) BY SUGARCANE  
(*SACCHARUM OFFICINARUM* L.) IRRIGATED WITH TREATED  
EFFLUENTS OF SUGAR INDUSTRY

USHA DAMODHARAN\*, M. VIKRAM REDDY

Department of Ecology and Environmental Sciences  
Pondicherry Central University  
Pondicherry – 605 014 India

\*Corresponding authors e-mail: ushadamodhar@gmail.com

**Keywords:** Trace metals, bioconcentration, transfer factors, sugarcane juice; treated effluent irrigation.

**Abstract:** The accumulation and translocation of trace metals in soil and in sugarcane crop irrigated with treated effluents from sugar industry compared to soil and sugarcane crop irrigated with bore-well water were determined. In the present study the impact of irrigation with treated effluent from the sugar industry on the trace metal contamination of sugarcane juice was assessed. It revealed that the mean concentrations of Cd, Pb, Cu, Mn and Zn in the soil of fields irrigated with effluent and in juice from sugarcane grown on such fields were higher than those from bore-well water irrigated fields. The concentrations of trace metals in treated effluent exceeded the permissible limits of the Indian standards (Central Pollution Control Board-2000). The concentrations of Cd, Pb, Cu and Zn in juice of sugarcane grown on fields irrigated with effluent also exceeded the permissible limits of Indian standards and WHO/FAO expert committee recommendations. Their concentrations in juice of sugarcane grown in fields irrigated with bore-well water were within the limits of safety, except for Cd. The transfer factor for Zn was considerably higher than those of the other trace metals. The metal concentrations of sugarcane juice showed significant correlations with those of soil, which was not the case when bore well water was used for irrigation.

## INTRODUCTION

Sugar industries discharge about 40 billion liters of effluents annually, being characterized by undesirable color, foul odor, high biochemical oxygen demand (BOD: 5000–8000 mg L<sup>-1</sup>), and chemical oxygen demand (COD: 25000–30000 mg L<sup>-1</sup>) (Bose et al. 2008). Treated industrial effluents are normally discharged onto open land or into nearby water bodies resulting in soil and ground water pollution. Sugar industry effluents are highly acidic and contain readily degradable organic matter. Diluted effluents can be used for irrigation without adversely affecting soil fertility. This improves the physical and chemical properties of the soil and increases the abundance of the soil microflora. The effluents contain nitrogen, phosphorous, potassium, calcium, and magnesium, and thus

they are valued as fertilizer. When applied to soil through irrigation, they are reported to increase the yield of sugar cane, rice, and wheat (Liu et al. 2007). However, long-term use of industrial effluents for irrigation may cause the concentrations of the trace metals Cd, Cu, Zn, Pb, and Mn to increase in surface soil, which may affect food quality and safety (Ibrahim et al. 1998). Bioaccumulation of certain trace elements (Cd, Pb, and Cu) in vegetables and fruits to toxic levels were related to high prevalence of upper gastrointestinal cancer (Abdulla et al. 1990).

The impact of effluent irrigation upon trace metal contamination in sugarcane has been studied for more than forty years, but none of the studies in India has addressed the risks of trace metal bioconcentration in sugar cane juice. Since the contamination levels of trace metals in the agricultural environment increase at alarming rates, the necessity for surveillance of consumer products such as sugar has prompted the present study. Thus, the aim of the present study is to highlight the contamination of juice extracted from sugarcane grown in effluent irrigated field with five trace metals, in relation to that of sugarcane irrigated with bore-well water. The trace metals were judged to have a potential for bioaccumulation in sugarcane juice and an assessment of the risks to human health when consuming the juice was attempted. This may provide a basis for controlling the irrigation with treated effluents, for improvement of the sugarcane quality, and may ultimately help to prevent exposure of humans to toxic metals. The knowledge of metal transfer factors allows predicting their potential uptake by plants in a particular area (Miller et al. 1992). In the present study it was investigated to which extent the concentrations of trace metals in sugarcane were correlated with their concentrations in treated sugar industry effluents used for irrigation and in soil.

#### TRANSFER FACTOR

Transfer factor describes the amount of an element expected to enter a plant from its substrate, under equilibrium conditions (Sheppard and Sheppard 1985; Davis et al. 1999). This theory assumes a linear relationship between the concentrations of a certain element in the plant with that in the soil. An approach based on soil-plant transfer factor provides an uncomplicated and helpful method for assessing uptake of trace metals for the purposes of assessment and testing. To portray quantitatively the transfer of an element from soil to plant, the soil-plant Transfer Factor (TF) of Concentration Ratio that expresses the ratio of contaminant concentration in plant parts to concentration in dry soil is used (Hope 1995; Roca and Vallejo 1995; Dinelli and Lombini 1996; Rodriguez et al. 2002; Tome et al. 2003). TF is calculated as follows:

$$TF = \frac{Con_{plant}}{Con_{soil}}$$

where, Con plant and Con Soil are the cane juice and soil concentrations, respectively. When a TF is < 1, it shows that the plant can only absorb but not accumulate trace metals, whereas when a TF is > 1, it shows that plant can accumulate metals (Bose et al., 2008). Transfer factor concept is the presence of a statistically significant relationship between the concentration of a given element in the soil and plant (Bunzl et al. 2000).

## MATERIALS AND METHODS

### *Study area*

Two sampling sites were selected based on their pattern of irrigation, i.e. the Nesanur farm (11°47'35.82" N and 79°33'46.88"E) was irrigated with sugar industry effluent (site 1), and the Lakshmi Narayana Puram farm (11°47'35.82" N and 79°33'46.88" E) was irrigated with bore well water (site 2) located at Cuddalore district of Tamil Nadu, on the southeast coast of India. Five adjacent plots (plot 1, 2, 3, 4, and 5), each located at a distance of approximately 5 m from the border of both sites, were selected for sampling and analysis.

### *Sampling and sample preparation*

Five samples of each, i.e. sugarcane, irrigation water, and soil from 0–15 cm depth made into composite samples, were collected from each of the five adjacent plots during the sugarcane growing period in monthly intervals from March 2010 to January 2011. Irrigation water samples were collected from site 1 and 2 in polyethylene cans of a capacity of 100 mL at monthly intervals. Approximately 1 mL concentrated HNO<sub>3</sub> was added to avoid microbial utilization of metals. Suspended particulate matter was separated by filtering the water samples through 0.45-mm Whatman GF/C filters. Filtered bore-well water and treated effluents were acidified to 0.5% (v/v) separately using concentrated nitric acid for the precipitation of samples. The soil samples were air-dried at room temperature, then pulverized, and sieved through a 1-mm stainless steel mesh (APHA 1995). Sugarcane samples were collected at the time of harvest with the aid of a stainless sharp knife. The cane stalks were rinsed with de-ionized water to remove dirt, then peeled, and cut to smaller sizes. The sugarcane juice was squeezed out into 500 mL plastic vials with the aid of a pre-cleaned extractor and kept refrigerated until analysis. Sugarcane juice samples of 5 mL were digested by addition of 15 mL of a mixture of concentrated HNO<sub>3</sub> (69%), H<sub>2</sub>SO<sub>4</sub> (98%), and HClO<sub>4</sub> (35%) (Merck, Darmstadt, Germany) in 5:1:1 ratio and were kept at 80°C until a transparent solution was obtained (Allen et al. 1998). The digested samples were filtered through 0.45 µm membrane filters and the filtrates were diluted to 50 mL with distilled and deionized water. Trace elemental concentrations of digested soil, water, and sugarcane juice samples were estimated by means of an atomic absorption spectrometer (GBC-AVANTA, Victoria, Australia).

### *Analytical Procedure*

Reagent grade chemicals and distilled deionized water were used throughout this analysis. Glassware was soaked with aqua regia for 2 hours and rinsed with distilled and deionized water. Standard stock solutions (1000 mg L<sup>-1</sup>) of Cd, Cu, Pb, Mn, and Zn (Reagecon, Shannon, Ireland) were diluted with distilled water into separate working standards of each metal in concentrations of 0.5, 1.0, and 1.5 mg L<sup>-1</sup> in 0.5 mol L<sup>-1</sup> HNO<sub>3</sub> (Cd, Zn), 1.0, 2.0, and 3.0 mg L<sup>-1</sup> in 0.5 mol L<sup>-1</sup> HNO<sub>3</sub> (Cu), 1.0, 2.0, and 3.0 mg L<sup>-1</sup> in 1.0 mol L<sup>-1</sup> HCl (Mn), and 4.0, 8.0, and 12 mg L<sup>-1</sup> in 0.5 mol L<sup>-1</sup> HNO<sub>3</sub> (Pb). Reagents' blank determinations were used to correct the instrument readings and the analyses were performed in three replicates.

### *Statistical Analysis*

All data for trace metals were represented as arithmetic means and SD. The variances among different samples were analyzed by two ways ANOVA and the correlation of

trace metals of the soil and sugarcane juice were shown in scatter plot diagrams. Two ways ANOVA and the other statistical analyses were performed using statistical package (SPSS-19.0). Variances were accepted when the probability of variance (P) was < 0.05. Bray cluster analysis was computed in bio diversity pro statistical package to interpret the data and to indicate the contaminant pattern.

## RESULTS AND DISCUSSION

The results of trace metal analysis of irrigated water, soil and sugarcane juice of five plots are shown in Figure 1. The trace metal concentrations in water were compared with CPCB (2000) standards and soil was compared with maximum permissible limits of Indian standards, and the metal concentrations in sugarcane juice were compared with maximum permissible limits as suggested by the joint FAO/WHO Expert Committee recommendations (2007) for Additives and Contaminants (JECFA) and Indian standards stated in Table 1.

### *Metal concentration in irrigated water*

The total concentrations (mg L<sup>-1</sup>) of Pb (0.764) and Zn (9.396) were several folds higher than the limit (<0.1 mg L<sup>-1</sup> for Pb and <5.0 mg L<sup>-1</sup> for Zn in surface water), while the total concentrations (mg L<sup>-1</sup>) of Cd (1.1), Cu (1.506) and Mn (1.8) were within the limit of CPCB (2000) standards (Table 2). This indicated that higher concentration of Pb and Zn

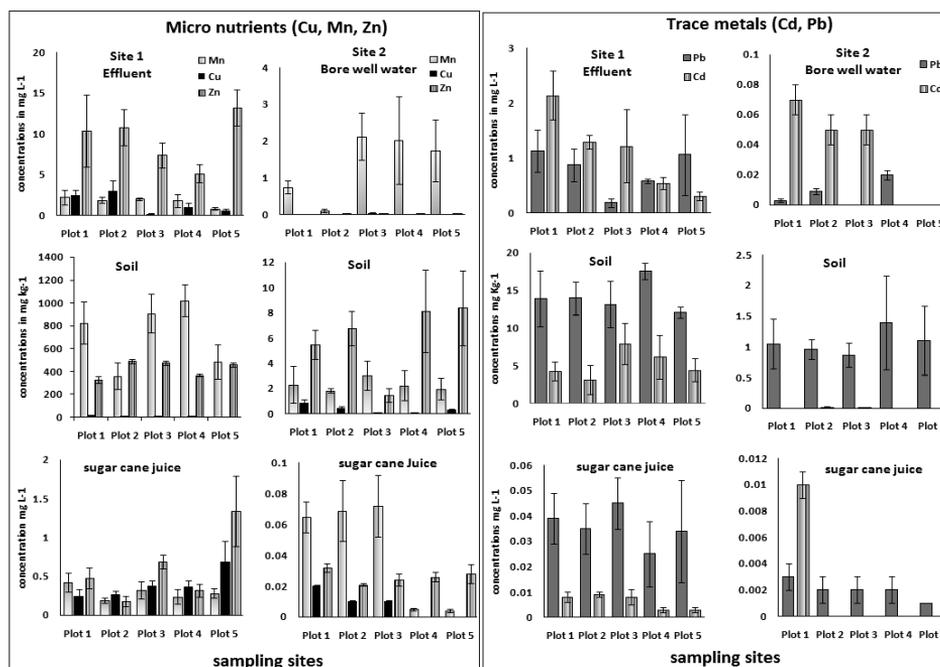


Fig. 1. Micro nutrient and trace metal concentration in treated effluents and irrigated water, soil and sugarcane juice of site 1 (effluent irrigated field) and site 2 (bore well irrigated field)

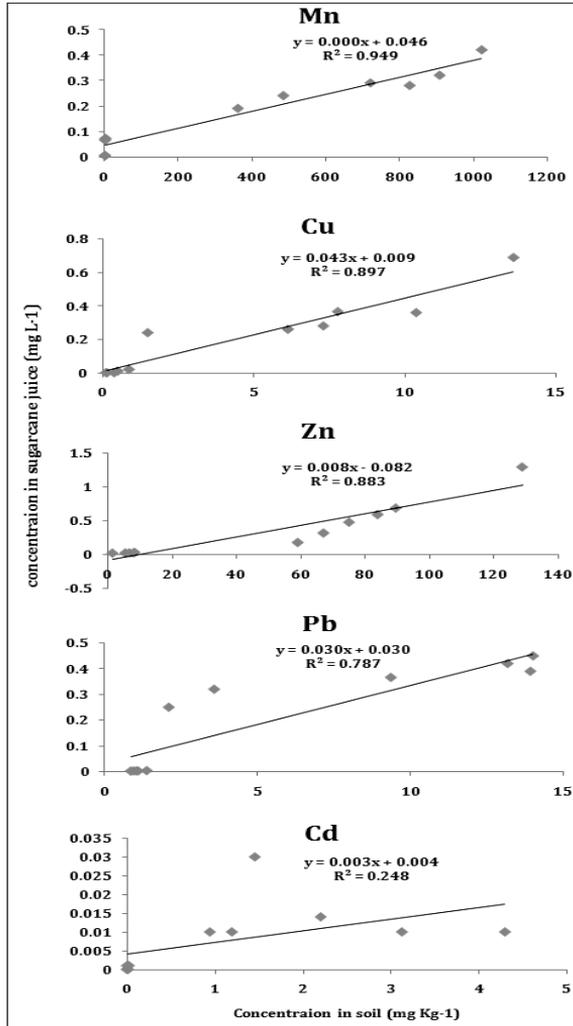


Fig. 2. Regression analysis of trace metal concentration in sugarcane juice and in soil of the effluent irrigated fields

Table 1. Concentration of various metals in working standard solutions

Metals	Purity	Concentrations ( $\text{mg L}^{-1}$ ) in working standards		
Cd	1000 $\text{mg L}^{-1}$ Cd in 0.5 $\text{HNO}_3$	0.5	1	1.5
Pb	1000 $\text{mg L}^{-1}$ Pb in 0.5 $\text{HNO}_3$	4	8	12
Mn	1000 $\text{mg L}^{-1}$ Mn in 1M HCL	1	2	3
Zn	1000 $\text{mg L}^{-1}$ Zn in 0.5 $\text{HNO}_3$	0.5	1	1.5
Cu	1000 $\text{mg L}^{-1}$ Cu in 0.5 $\text{HNO}_3$	1	3	5

was due to the contemporary mechanized agriculture which was consistent with Taylor and Percival (2001). Large-scale applications of industrial effluent that exceeds their metal toxicity limits can have deleterious effects on sugarcane growth (Anon 1992–1993). The excessive Zn concentration in water is of great concern because of its toxicity to humans and animals (Kabata-Pendias and Mukherjee 2007). Industrial effluents containing trace metals also pollute cultivated fields and underground water through seepage. The levels of Pb can range from 2 to 200 ppm in such agricultural lands (Anon 1992–1993). However, the concentrations of all metals in bore-well water were within the prescribed limit and concluded to be safe for irrigation.

Table 2. Guideline for safety limits of trace metals

Sample	Standards	Cd	Cu	Pb	Zn	Mn
Water (mg L <sup>-1</sup> )	Indian Standards (Awashthi 2000)	0.001	0.05	0.10	5.0	0.10
	CPCB (2000)	2	3	0.1	5.0	2.0
Soil (mg kg <sup>-1</sup> )	Indian Standards (Awashthi 2000)	<0.006	<0.27	<0.5	<0.6	–
	FAO (1985)	0.01	0.20	5.0	2.0	0.20
Plant (mg L <sup>-1</sup> )	Indian Standards (Awashthi 2000)	0.001	0.03	0.0025	0.05	–
	WHO/FAO (2007)	0.002	0.04	0.005	0.06	–

### ***Metal concentration in soil***

The mean concentrations of Cd, Cu, Pb, Zn and Mn in site 1 and 2 soil are shown in Figure 1. The total concentrations (mg kg<sup>-1</sup>) of soil Cd (5.198), Cu (7.766), Pb (14.154) and Zn (424.0) at site 1 were several folds higher than the Indian standards stated in Table 2 (Awashthi 2000). Whereas the total concentration (mg kg<sup>-1</sup>) of soil Cu (0.392), Pb (1.076) and Zn (6.044) at site 2 exceeded the Indian standards except Cd (0.006). Cd can accumulate in agricultural soils through the application of soil amendments like phosphatic fertilizers and sewage sludges, which are known to contain Cd levels of 7.3–170 mg kg<sup>-1</sup> (Lisk 1972). The repeated use of Bordeaux sprays can cause Cu toxicity in soil and plants (Reuther and Smith 1954). Despite of metal contamination from treated industrial effluents, other chemicals ordinarily used in agriculture, such as chemical fertilizers, pesticides, manure, limestone, as well as urban wastes (sewage sludge and fly ash) contain significant quantities of heavy metals such as Cu, Zn, Cd and Pb which became the source for metal contamination in soil (Foy et al. 1978).

### ***Metal concentration in sugarcane juice***

Sugarcane (*Saccharum* spp.) has the potential to be a phytoremediator species due to its outstanding biomass production (Serenio et al. 2007). The mean concentration of trace metals in the sugarcane juice (Figure 1) showed that all the studied metals were

accumulated in sugarcane collected at site 1 and 2. However, the total concentration ( $\text{mg L}^{-1}$ ) of Cd (0.006), Cu (0.4), Pb (0.04) and Zn (0.6) at site 1 exceeded the Indian and WHO standards (Table 2). However the total concentration ( $\text{mg L}^{-1}$ ) of Cd at site 2 was one fold higher than the Indian standards and several folds higher than the WHO standards. Trace metals present in the soil are absorbed by the plants growing in it to either sufficient or phytotoxic levels (Dhillon and Dhillon 1996). Internal concentrations of Cd and Zn were more influenced by the soil/environment than by the variety of sugarcane, while the distribution of metals in plant parts was quite consistent. About 77% of the Cd and 56% of the Zn were contained in the stem (Rayment et al. 2002). Sugarcane exhibits tolerance to 500  $\mu\text{M}$  Cd and 100  $\mu\text{M}$  of Cu without exhibiting any symptoms of toxicity; it accumulates 451  $\text{mg kg}^{-1}$  shoot dry weight, indicating its potential as a Cd phytoremediator (Serenio et al. 2007). High concentrations of soil Pb reduced the sugarcane's uptake of most other nutrients due to damaging effect on the root (Mengel 1978). Barzegar et al. (2005) reported that Cd is accumulated in bagasse and Pb is primarily accumulated in bagasse and molasses. As a result, the levels of these trace metals in cane juice are lower at uncontaminated site. The present results were in agreement with previous studies.

The concentrations of Cd, Pb, Mn, Cu and Zn in water, soil and juice of the two sampling sites were with the following ranking pattern – soil > water > juice extract. The present results showed the lesser trace metal concentration of water, soil and sugarcane juice in site 2 because of the lesser metal contamination of the bore well water (Singh et al. 2011).

Transfer factor (TF) was calculated as the ratio of content of trace metal in the plant or the part of plant to that in the water and soil. The TF values for Pb, Cd, Cu, Mn and Zn in sugarcane plant are shown in Figures 3(a) and (b), which estimates the Transfer Factor of different heavy metals from water and soil to vegetation. The TF values for trace metals varied greatly between irrigated water to crop and soil to crop ratio. The transfer factor of Mn, Pb, Cd, Cu and Zn in sugarcane juice from irrigated water and soil of both the sampling sites were lesser than one. One of the reasons for these results is that the plant roots damaged by Pb contamination in soil become weaker in absorbing the other nutrients (Mengel 1978). The highest TF values were found for Cu, Pb and Zn from irrigated water to sugarcane, because these metals are more portable in nature. The highest TF values for Cu, Cd and Pb from soil to crop showed a strong accumulation of Cd and Zn by sugarcane.

Bray Cluster Analysis (CA) was applied to detect the metals of similar sources of effluent and bore well irrigated field. The dendrogram depicted 4 clusters among the 5 metals of sampling sites. It showed that in both sampling sites Pb and Cu formed one cluster with 83% similarity to which Cd joined with 49% similarity indicating that these metals are from the similar sources (Fig 4). Zn and Mn formed a distinct separate cluster with 72% similarity indicating that these metals are from the similar sources but different from the other three metals (Pb, Cu and Cd). Accumulation of trace metals (Cd, Pb, Cu, Mn and Zn) in the soil due to the use of effluent irrigation represented a risk for the soil productive potential and incorporation of metals into the food chain. However, the result of this investigation put in evidence that irrigation with wastewater should not be carried out for unlimited period, since trace metals tend to accumulate in the soil and subsequently can be absorbed by the crops.

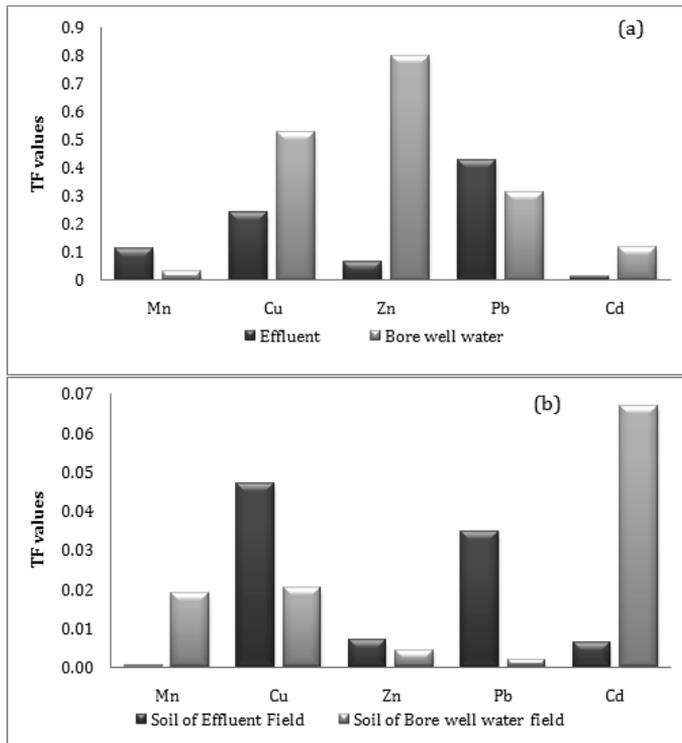


Fig. 3. TF values of Mn, Cu, Zn, Pb and Cd in sugarcane juice grown in effluent and bore well irrigated fields: (a) water (b) soil

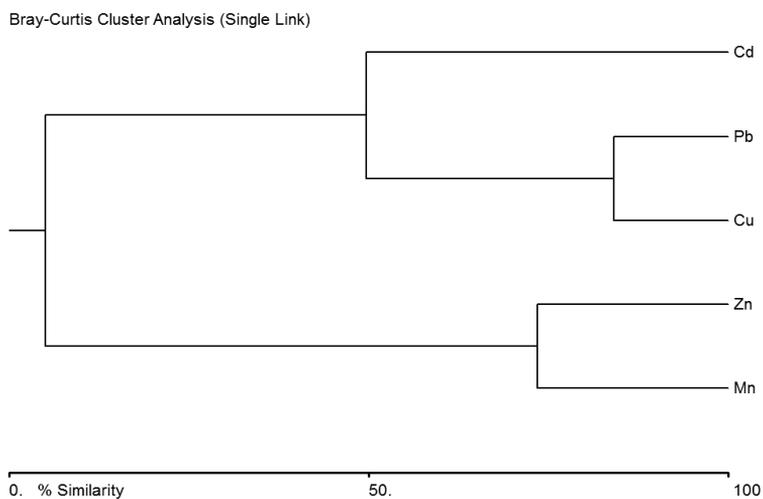


Fig. 4. Bray-Cluster Analysis (Single Link)

## CONCLUSION

Trace metals accumulation in soils not only exerts deleterious effects on plant growth, but also affects the soil fertility and microbial communities of soil. Metal accumulation in agricultural soils together with associated naturally eroded metals will remain a chronic trace metal pollution problem. There is a serious health risk posed by cultivation of sugarcane that receives trace metals from irrigation of industrial effluents as well as application of chemical fertilizer. The exceeding level of trace metals in juice extract may also be due to direct wet and dry deposition of pollutants on soil and foliage, which can hardly be ignored as possible sources of the metals in the sugarcane plant juice and consequently sugar. The local population needs to be educated through awareness programs of using the treated effluents in agriculture including sugarcane and the possible dangers inherent in the consumption of the sugarcane cultivated in these areas.

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