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Assessment Of Type 2 Diabetes Mellitus Risk Associated with Zinc Accumulation in Ethnic Millets from Zawar Mines, Udaipur (Raj.), India

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Abstract

Objectives: Operative cum post-mining procedures make many health issues as they enter food chains and causes bio-magnification. Zinc en-routes through rhizosphere, roots, shoots and finally in grains. This study was conducted to assess the zinc levels in soil, its translocation in seven millet plants grown near the area of Zawar Mines, Udaipur i.e. *Echinochloa colona*, *Echinochloa crusgalli*, *Eleusine coracana*, *Panicum miliaceum*, *Paspalum scrobiculatum*, *Setaria glauca* and *Setaria italic*, and the impact of higher zinc accumulated grains on postprandial hyperglycemic biomarker enzymes, specifically α-amylase and α-glucosidase, total sugar content and total dietary fiber, considering the role of zinc as a micronutrient involved in regulation of oxidative stress and in glucose metabolism. **Methods:** Metallic bioaccumulation and translocation was assessed by three parameters viz. bio-concentration factor, bio-accumulation factor and translocation factor. Ethno anti-hyperglycemic millets were screened for four parameters viz. total sugar content, total dietary fiber, α -amylase and α -glucosidase inhibitory activity. **Results:** The zinc concentrations were higher in grains of the millets except for *E. colona* and *E. crusgalli*. The risk assessment for diabetes mellitus II reveals low levels of total sugar content in test samples. Total dietary fiber didn't reveal significant variation among control and test samples. Anti α-amylase and anti α-glucosidase activity were comparatively higher in test samples of *E. colona* and *E. crusgalli* but only *E. colona* marked as safer for diabetic patients as grains of *E. crusgalli* had high levels of zinc, more than ingestible permissible limits. **Conclusion:** Zinc content in ethnic millets significantly influence the risk of Type 2 Diabetes Mellitus. The findings suggest that millets containing adequate levels of zinc may contribute to improved α-amylase and α-glucosidase inhibitory activity, while lower absorbed zinc levels due to poor bioavailability may increase susceptibility to the disease.

Keywords

Zawar Mines, Type 2 Diabetes Mellitus, Ethnic Millets, Bioaccumulation factor, Translocation factor.

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INTRODUCTION

In nutrition, various components form different pillars of health among which minerals are of utmost importance as they are the basic keys of various enzymatic functions. These minerals form a balanced metabolic entity between environment and human health but in mining zones and industrial areas, these entities have deviated from ideal levels leading to hazardous impacts [1-4]. Metal is present in the soil in various forms—specially, exchangeable, oxidizable, reducible and residual forms. Among these, only the exchangeable metal fraction contributes to metal mobility and is bioavailable to plants [5-8]. Rajasthan is rich in terms of mining zones and ores of various mineral deposits. The Zawar group of mines are rich in Lead –Zinc ore deposits and are situated in Rajasthan, India, approximately 40 km south of Udaipur. The zinc moiety in this region varies invariably and affects human health through both direct and indirect ways [9,10]. Many plants growing in mining vicinity are bio-accumulators and indicate high levels of zinc in roots, shoots and grains when compared with control or non-mining sites. When such plants are ingested by the local populace for their ethno-botanical usage, they affect many biological pathways. Tribal culture predominantly denies the practice of stored foods except for staple grains. Among grains, they prefer millets over cereals due to their economical and easy availability [11-14].

Cereals and millets despite complex sugar entities carry a good amount of fiber which in turn affects satiety, a major cause of obesity and in turn affecting DMII by hindering obesity [15]. Therefore, in the present investigation the millets chiefly consumed by ethnic peoples were prioritized over other plant materials and seven millets *Echinochloa colona*, *E. crusgalli*, *Eleusine coracana*, *Panicum miliaceum*, *Paspalum scrobiculatum*, *Setaria glauca* and *S. italica* were used as test samples.

Type 2 Diabetes Mellitus (DMII) describes a category of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. These persistent higher sugar levels specifically higher postprandial sugar levels are the basic cause of diabetic-associated multiple pathologies. Amylase and glycosidase are classified in the largest Glycosyl hydrolase family, GH13 [16-17]. They are the key carbohydrate degrading enzymes, help in breaking down of polysaccharides into smaller molecules and contribute to elevated blood glucose levels and postprandial hyperglycemia. Natural α-amylase inhibitors of plant origin have gained popularity as alternatives as they are safe and cost-effective and can manage diabetes mellitus II [18-19]. Transition metals are known to restrict α-amylase function by inducing changes to their native protein conformation [20].

Depending on its concentration, Zn can serve as an essential micronutrient [21,22], or can act as a hazardous heavy metal toxin [23-25]. It is estimated that zinc is a vital co-factor for about 3,000 enzymes and plays vital role in plant's defence system by quenching free radicals, and thereby reducing ROS damage to cell components [26,27]. Zn often acts as a protein cofactor by binding directly with the oxygen, sulphur, and nitrogen atoms present in the side chains of specific amino acids: aspartate, cysteine, glutamate and histidine [28] and has significant impact on every aspect of a person's metabolic processes and overall health [29]. The damaging consequences of heavy metals are largely associated to their capability to interfere with free radical scavenging mechanisms and metabolic pathways [30]. Essential nutrients, such as zinc, selenium, and other can reduce heavy metal bioavailability and toxicity [31,32]. Zinc causes the chelation and immobilization of heavy metals which in turn prevents their absorption by plants from the soil [33]. At optimum concentration Zn mitigates heavy metals toxicity with hyper- accumulators' capacity through the over expression of constitutive metal transporters and their efficient translocation capacity [33-34]. It also induces the synthesis of metallothioneins, the cysteine-rich heavy metal binding proteins, which effectively immobilize zinc ions and prevent them from interfering with cellular functions [35]. Zinc plays important roles in the adequate insulin synthesis, secretion and its translocation into the cells. In addition, zinc activates phosphoinositol-3-kinase and suppress pro-inflammatory cytokines [36].

Excess Zn in the soil can interfere with the absorption and movement of other essential micronutrients and can directly harm cell membrane and organelles [23]. It can disturb nutrient homeostasis and other vital physiological activities. Its presence above threshold limit triggers oxidative stress, cellular damage and alteration in the structure and functions of enzymes, resulting in a high generation of free radicals [27,30]. Thus, an adequate amount of zinc is essential for structural, catalytic and regulatory functions of various enzymes and their stability [37-39].

Zn uptake in plants is determined by its availability in the soil and the plant's ability to absorb and manage its internal water and mineral levels [40]. Naaz and coworker, explained the mechanism of Zn absorption from the soil, role of transporter proteins and factors governing zinc availability in the soil [41]. Numerous studies demonstrated that free radicals play a significant role in insulin resistance and diabetes mellitus [42]. En-route of zinc in humans through contaminated soils via grains can impact sugar levels in diabetic patients [10]. Exposure to hazardous metals from unregulated mining and industrialization, is known to contribute to oxidative stress and related diseases. However, there is currently no evidence linking heavy metals with type 2 diabetes, with the exception of arsenic [43]. Previous studies have examined the effect of varying zinc levels on tolerance, accumulation capacity, and stress responses in different millets species [41,44]. Building on this line of research, the purpose of the current *in vitro* study was to assess the zinc levels in soils, its translocation within millet plants and the impact of higher zinc- accumulated millet grains on postprandial hyperglycemic biomarker enzymes i.e.α-amylase and α-glucosidase, total sugar content and total dietary fiber. Such studies on health risk assessment of dietary exposure to metallic trace elements like lead, cadmium and other toxic elements support continuous monitoring of toxic elements in staple foods, strengthen the management of food safety and support the creation of national policies [45-50].

2. MATERIALS AND METHODS

2.1 Study area

The Zawar Belt with zinc- lead deposits (Mochia, Balaria, Zawar Mala, Baroi and Bawa) situated within the Lower to Middle Proterozoic Aravalli Super groups, forming a 20 Km long mineralized zone that includes intercalated dolomite, quartzites and conglomerates, geographically spread around 24° 18' 48" and 24° 22' 48" N and 73° 40' and 73° 45'24" E in Rajasthan [51]. A tropical continental monsoon is the prevailing climate type of the area. (Plate 1).

Plate 1: Zawar Belt with zinc lead deposits at Mochia, Balaria, Zawar Mala, Baroi and Bawa (24° 18' 48" and 24° 22' 48" N and 73° 40' and 73° 45'24" E)

2.2 Sample collection

Rhizospheric soil samples were taken from the Zawar Belt with zinc- lead deposits Mochia, Balaria, Zawar Mala, Baroi and Bawa. The millet species i.e. *E. colona* (EC1), *E. crusgalli* (EC2), *E. coracana* (EC3), *P. miliaceum* (PM), *P. scrobiculatum* (PS), *S. glauca* (SG) and *S. italica* (SI) were gathered from 17 locations surrounding the mining and land fill area. Additionally, a control soil sample was also collected from a forested conserved land that was unaffected by mining activities. Metal contaminated free millet plant species were collected from mining deficit areas which were used as control. Soil samples and plant specimens were transported to the laboratory after being placed in pre-cleaned, sealable, airtight bags. Millets plant specimens (Herbarium Ac. No. MC/MS 110-124/2023) were identified by Prof. S. S. Katewa, Department of Agrostology and Ethnobotany, MLSU, Udaipur (Raj.) and are kept in Department of Botany, B N University, Udaipur (Raj.) for future reference.

2.3 Sample preparation and analysis

Zinc was analyzed according to Ure, 1990 [52]. The soil samples were oven-dried at 100° C for 24 hours, ground to a fine powder and sieved through a sieve of 2.0 mm mash size to get rid of impurities from the samples. Vegetative millet plant materials were initially washed for the removal of dust and other adhered impurities. Later on, they were washed with deionized water and pressed over filter papers to remove moisture and subsequently seeds were ground and sieved to obtain fine powder for zinc analysis. 1.0 g of each sample were taken in 250 ml glass beakers and digested with concentrated nitric acid and perchloric acid by heating the mixture of samples on a hot plate in a fume cupboard at 200°C. The volume of sample solutions and control was made up to 10.0 ml with deionized water. Millets/Grains were analyzed for zinc by oven drying at 105̊ C for two hours, subsequently cooled to room temperature and ground to a fine powder. 1 g of sample was turned to ash at 450̊ C and digested to clear solution with 5 ml of HNO_3. The residue was made to 50 ml by adding deionized water. Metal content in an analyte was determined using atomic absorption spectrophotometer. Metallic bioaccumulation and translocation were assessed by three parameters viz. bio-concentration factor (BCF), bioaccumulation factor (BAF) and translocation factor (TF) [53-55].

i. $BCF = \frac{C_{root}}{C_{soil}}$

ii. $BAF = \frac{C_{shoot}}{C_{soil}}$

iii. $TF = \frac{C_{shoot}}{C_{root}}$

C_{root}, C_{shoot} and C_{soil} are metal concentrations in the plant root, shoot and rhizosphere respectively. The concentrations are expressed as (mg /kg). TF >1 depicts that translocation of metals was functionally made to the shoot from the root. BAF is further represented as hyper-accumulator, accumulator and excluder to plant species which accumulates metals as >1, 1 and <1, respectively.

2.4 *In vitro* biochemical evaluation for anti-diabetic potential

Ethno anti-hyperglycemic millets were screened for their anti-diabetic potential for four parameters viz.- Total Sugar Content (TSC), Total Dietary Fiber (TDF), Anti α-amylase Activity (AAA) and Anti α-glucosidase Activity (AGA). For *in vitro* studies, the aqueous extract was prepared by cold maceration of respective grains by soaking 100g in 500ml of distilled water for 24 hours and followed by filtration. Respective extracts were stored at 5-8̊ C in dark bottles.

Total sugar content

Carbohydrates were first hydrolysed into simple sugars using dilute hydrochloric acid. The millet samples were weighed (100 mg) and into boiling tubes, HCl (2.5 N, % ml) was added and the tubes were kept in a boiling water bath for 3 hours for hydrolysis. The tubes were cooled to room temperature. Sodium carbonate was added to the samples until the effervescence ceased. This neutralized the samples. The volume was made up to 100 ml and centrifuged. The supernatant was collected and 0.1 ml aliquots were taken for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.5, 0.8, and 1 ml of the working standards. Zero (0) served as blank. The volume was made up to 1 ml in all the tubes including the sample tubes by adding distilled water. To all tubes 4 ml anthrone reagent was added. The tubes were heated for eight minutes in a boiling water bath. The contents of the tube were cooled rapidly and the dark green colour was read at 630 nm. A standard graph was drawn by plotting the concentration of the standard on the X-axis versus absorbance on the Y-axis.

$$\textbf{Total sugar content} = \frac{\textbf{mg of glucose X 100}}{\textbf{Volume of the test sample}}$$

Total dietary fiber

Total dietary fiber was screened using a combination of enzymatic and gravimetric methods (AOAC International, 1997). Fat-free dried samples bran were gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase enzymes to remove the protein and starch present in the sample and subsequently ethanol was added to precipitate the soluble dietary fiber. The residue was washed with ethanol and acetone and was dried and weighed. One portion of the sample was analysed for protein while the other part was turned to ash using muffle furnaces. TDF was calculated as the weight of the residue minus the weight of the protein and ash reported as a percentage of the original sample weight.

$$\textbf{TDF\%} = \frac{[(\textbf{R Sample} - \textbf{P Sample} - \textbf{A Sample}) - \textbf{B}] * \textbf{100}}{\textbf{S}}$$

Where, Residue weight (mg) (R) = W2-W1; Ash weight (mg) (A) = W3-W1; B = R (blank) – P (blank) - A (blank); P = Protein weight (mg) and S = Sample weight (mg).

Anti α-amylase activity

The assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme and the aqueous millet extract in the concentration range 20-100 µgml^{-1} were incubated for 10 minutes at room temperature followed by the addition of 200 µl of starch in all test tubes. The reaction was terminated with the addition of 400 µl DNS (3,5-Dinitrosalicylic acid) reagent and placed in a boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any millet extract. The % inhibition was calculated according to the formula [56].

$$\textbf{Inhibition}\ (\%) = \frac{\textbf{Abs}_{\textbf{540}}\ (\textbf{control}) - \textbf{Abs}_{\textbf{540}}\ (\textbf{extract}) * \textbf{100}}{\textbf{Abs}_{\textbf{540}}(\textbf{control})}$$

Anti α-glucosidase activity

The yeast alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Aqueous millet extracts were used in the concentration ranging from 20-100 $\mu g ml^{-1}$. Different concentrations of millet extracts were mixed with 320 µl of 100 mM phosphate buffer pH 6.8 at 30°C for 5 minutes. 3 ml of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without any extracts. The % inhibition was calculated according to the formula [56].

$$\textbf{Inhibition}\ (\%) = \frac{\textbf{Abs}_{\textbf{410}}\ (\textbf{control}) - \textbf{Abs}_{\textbf{410}}\ (\textbf{extract}) * \textbf{100}}{\textbf{Abs}_{\textbf{410}}(\textbf{control})}$$

The IC 50 values for both α-amylase and α-glucosidase were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by nonlinear regression analysis from the mean inhibitory values. Acarbose was used as the reference α-amylase and α-glucosidase inhibitor.

2.5 Statistical analysis

The data obtained in triplicate were subjected to ANOVA at a significance level of P<0.001, 0.01 and 0.05.

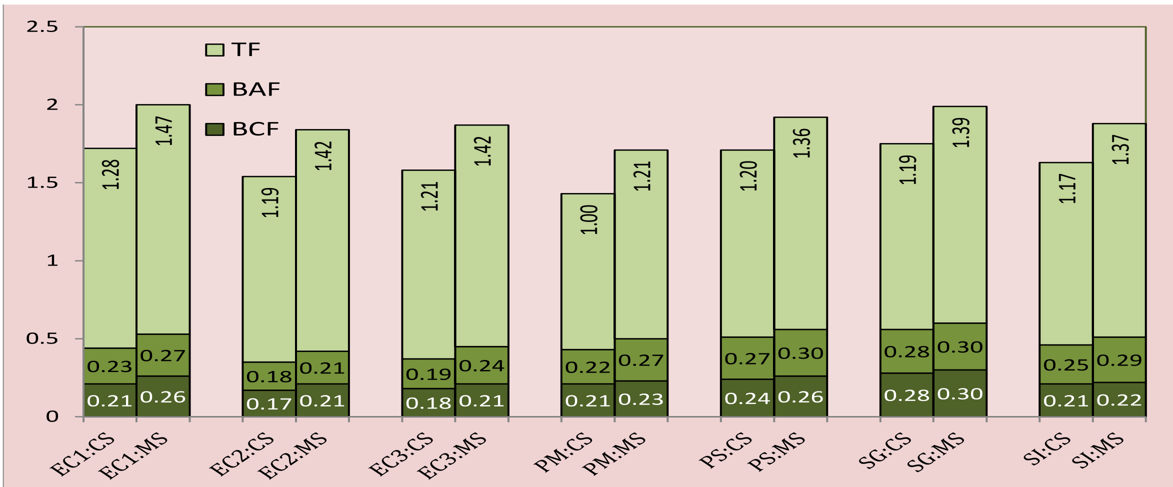
3. RESULTS AND DISCUSSION

In the current era, the soil is continuously contaminated through various anthropogenic activities [57]. In mining sites, bio-magnification of metals in plants of adjoining areas is a common observation and their channelization through food chain causes alarming clinical pathologies among which endocrine maladies are of utmost prominent [58].

Rajasthan state inhabits a large number of nomadic, seminomadic, tribal and rural population which resides in remote areas and depends on ethno-foods for their survival among which millets are more popular.

3.1 Bio-concentration factor (BCF), Bioaccumulation factor (BAF) and Translocation factor (TF):

Zinc concentration in soil samples and rhizosphere was between 27 to 30 $mg kg^{-1}$ in control sites while 35.5 to 36.87 $mg kg^{-1}$ in mining sites. Highest bio-concentration factor of zinc in control sites was obtained in *S. glauca* (0.28) followed by *P. scrobiculatum* (0.24) whereas in mining sites, similar to the control site both the species had similar higher concentration but in addition, *E. colona* had comparatively higher accumulation in mining site as compared to control site revealing significant bioaccumulation in root tissue. Comparison of bio-concentration factor (BCF) in between control sites and mining sites reveals a higher preference of 23.81% accumulation in *E. colona* followed by 23.53% in *E. crusgalli* and 16.67% in *E. coracana.* BCF was comparatively lower in *P. miliaceum, P. scrobiculatum*, *S. glauca* and *S. italica* and were in the range of 4 to 9 %. The zinc content in roots and shoots was nearly similar in *S. glauca* whereas it was 12.50 % more in *P. scrobiculatum* in the mining site as compared to the control site. Bioaccumulation factor (BAF) was significantly high in mining sites i.e.7.14 to 26.32% over control site as being higher in *E. coracana* (26.32%) followed by *P. miliaceum* (22.73%) and *E. colona* (17.39%) while lowest in *S. glauca* (7.14%). Translocation factor (TF) was found to be higher in all the studied millet species in the mining site by 13.33 to 21%. TF was found to be 21% higher mining site as compared to control in *P. miliaceum* followed by 19.33% in *E. crusgalli*. Change in TF over both the sites was nearly similar in *E. colona*, *E. coracana*, *S. glauca* and *S. italic* (Figure 1). BAF values of all the millets were less than one indicating their excluder nature but the values were still higher up to 24% in crops growing in mining adjoining fields as compared to control sites. In addition, higher TF values specifically of *P. miliaceum* and *E. crusgalli* indicates accumulation of zinc in significant quantities in aerial parts. Higher bioaccumulation of zinc in staple food/ millets and their daily consumption over long periods can cause chronic diseases [59].



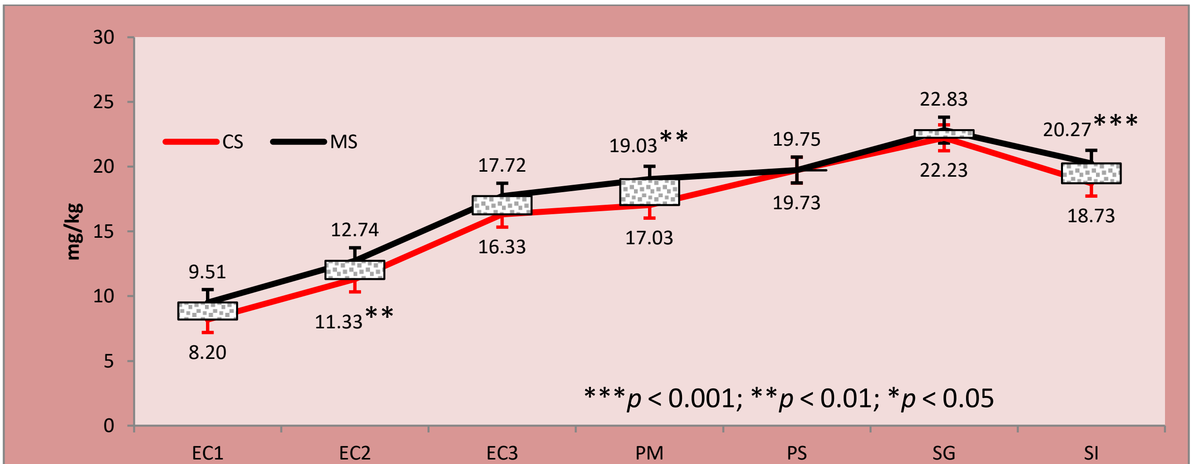
Echinochloa colona (EC1), *Echinochloa crusgalli* (EC2), *Eleusine coracana* (EC3), *Panicum miliaceum* (PM), *Paspalum scrobiculatum* (PS), *Setaria glauca* (SG) and *Setaria italica* (SI)

Figure 1: Comparison of Bioconcentration factor (BCF), Bioaccumulation Factor (BAF) and Translocation Factor (TF) of studied millets in control sites (CS) and Mining sites (MS)

3.2 Zinc concentration in grains:

Zinc concentration differed in grains of all the studied millets except in *P. scrobiculatum* in which the concentration was nearly similar i.e. 19.73 and 19.75 mgkg^{-1} in both control and mining site. Zinc concentration was higher up to 16.02% in grains collected from mining sites in *E. colona* followed by 12.41% and 11.74% in *E. crusgalli* and *P. miliaceum* respectively (Figure 2).

Higher accumulation of zinc in grains in mining site may lead to chronic maladies and impairment of organs passively leading to endocrine dysfunctions as the Recommended Dietary Allowance of zinc for adults is 11 mg per day for men and 8 mg/ day for women while the tolerable upper intake level for adults is 40 mg per day, a value based on reduction in erythrocyte copper-zinc superoxide dismutase activity [30, 60]. Nearly 100 specific enzymes (e.g., EC 1.1.1.1 alcohol dehydrogenase) depend on zinc for their catalytic action. Zinc deficiency impairs various activities including major biochemical pathways regulated through metalloenzymes [18,61,62]. Higher intakes of 150–450 mg of zinc per day have been associated with chronic effects as low copper status, altered iron function, reduced immune function, and reduced levels of high-density lipoproteins [63]. Reductions in a copper-containing enzyme, a marker of copper status, have been reported with even moderately high zinc intakes of approximately 60 mg/day for up to 10 weeks [30,64].

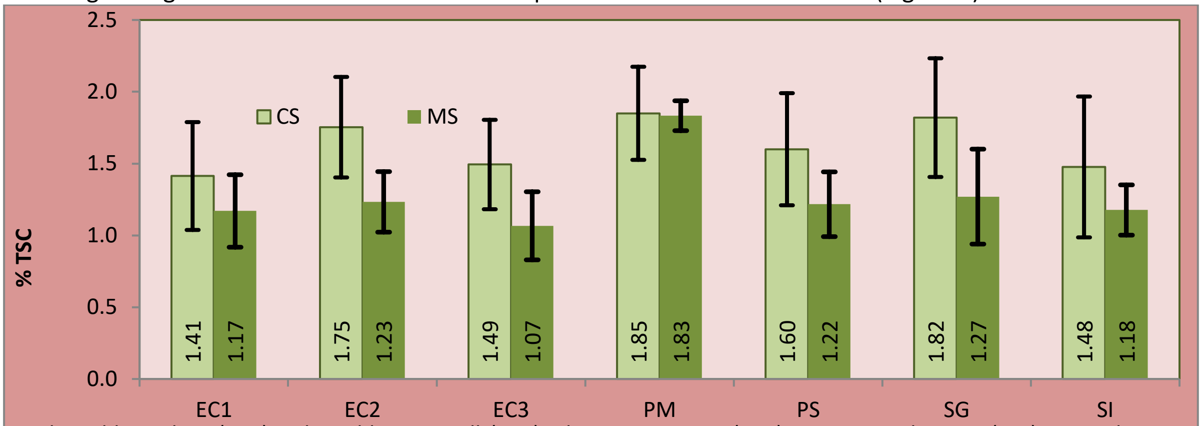


Echinochloa colona (EC1), *Echinochloa crusgalli* (EC2), *Eleusine coracana* (EC3), *Panicum*

Figure 2: Comparison of Zinc concentration in grains of studied millets at mining site (MS) and control site (CS)

3.3 Total sugar content (TSC) and Total dietary fiber (TDF)

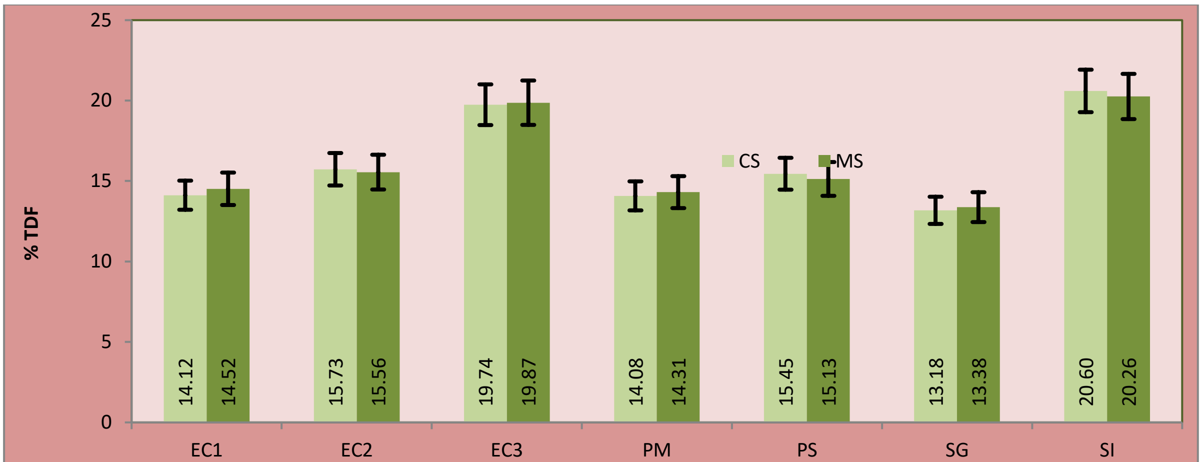
Total sugar content (TSC) was found to be lower in all millets growing in zinc contaminated soils except in *P. miliaceum* in which the TSC in grains differed only by 0.90% in control and test samples. TSC decreased by 30.22% in *S. glauca,* 29.66% in *E. crusgalli* and 28.57% in *E. coracana* (Figure 3).



Echinochloa colona (EC1), *Echinochloa crusgalli* (EC2), *Eleusine coracana* (EC3), *Panicum miliaceum* (PM), *Paspalum scrobiculatum* (PS), *Setaria glauca* (SG) and *Setaria italica* (SI); ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

Figure 3: Comparative % total sugar content (TSC) in grains of studied millets in mining site (MS) and control site (CS)

Total Dietary Fiber (TDF) did not differ largely in all test samples as compared to control samples revealing no effect of zinc concentrations in soil and was not affected by translocation factor. TDF was found to be slightly higher in *E. colona* (2.82%), *P. miliaceum* (1.65%) and *S. glauca* (1.51%) at zinc contaminated mining sites while decreased slightly in *P. scrobiculatum* (2.09%) followed by and *S. italica* (1.68%) and *E. crusgalli* (1.13%) (Figure 4).



Echinochloa colona (EC1), *Echinochloa crusgalli* (EC2), *Eleusine coracana* (EC3), *Panicum miliaceum* (PM), *Paspalum scrobiculatum* (PS), *Setaria glauca* (SG) and *Setaria italica* (SI); ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

Figure 4: Comparative % total dietary fibre (TDF) in grains of studied millets in mining site (MS) and control site (CS)

Prevailing beliefs over the past regarding sugars and diabetes admonished that simple sugars would confer a higher postprandial glycemia than starch, as different types of sugars may have variable metabolic effects on glycemia or lipemia [65]. Consequently, diets for diabetic patients have been sugar restricted for fear of stimulating hyperglycemia, exaggerating insulin response to carbohydrates and causing possible cardiomyocyte dysfunction and/or accelerated loss of β-cells [66]. Various studies concluded that fibers intake and obesity are inversely correlated, indicating their role in starch and cholesterol control [67-68]. Reduction in total sugar content and persistence of dietary fibers in zinc contaminated sites may help in regulating and managing glycemic levels in DMII patients [43,69,70].

3.4 Anti α-amylase activity (AAA) and Anti α-glucosidase activity (AGA)

Highest AAA was obtained in *S. italica* as 47.63% in CS and 48.72% in MS followed by *E. coracana* as 45.66% in CS and 46.13% inhibition in MS but comparison of inhibitory activity on both CS and MS didn't vary significantly revealing neutral impact of zinc concentrations in plant parts and rhizosphere while comparison of activities in CS and MS reveals increased AAA by 8.57% in *E. colona* and 7.48% in *S. glauca* (Figure 5). As akin to the activity of AAA, AGA was also found to be increased by 11.84% and10.77% in both *E. colona* and *S. glauca* while was nearly equal on both CS and MS in *S. italica* while contrary to all other millets responses AGA of *Eleusine* decreased sharply in zinc contaminated site by 26.86% (Figure 6).

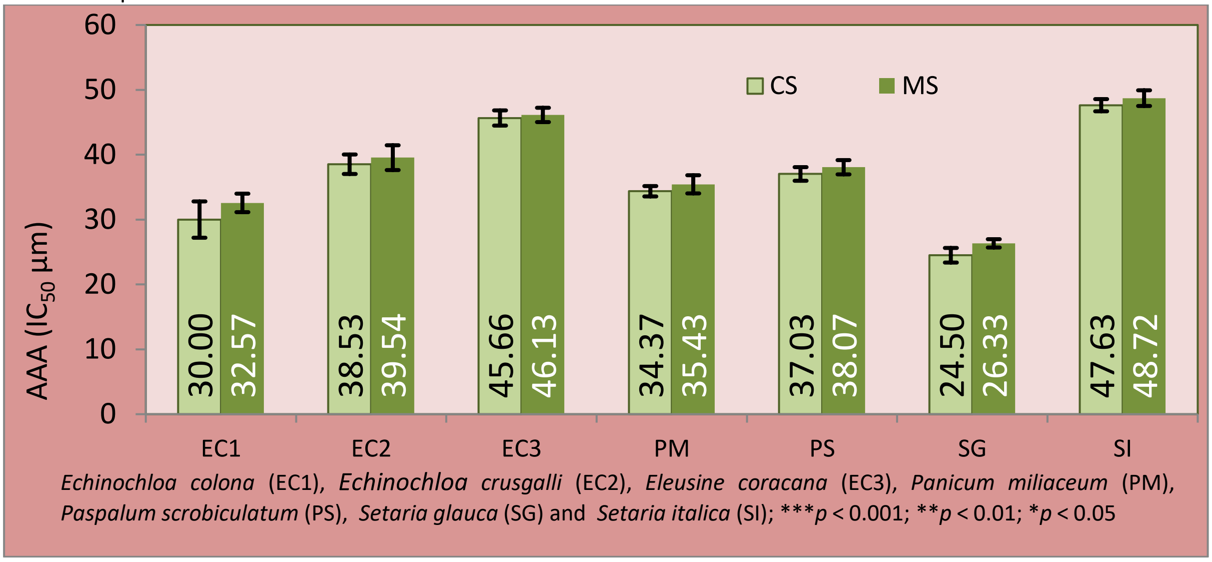


Figure 5: Comparative Anti α-amylase activity (AAA) in grains of studied millets in mining site (MS) and control site (CS)

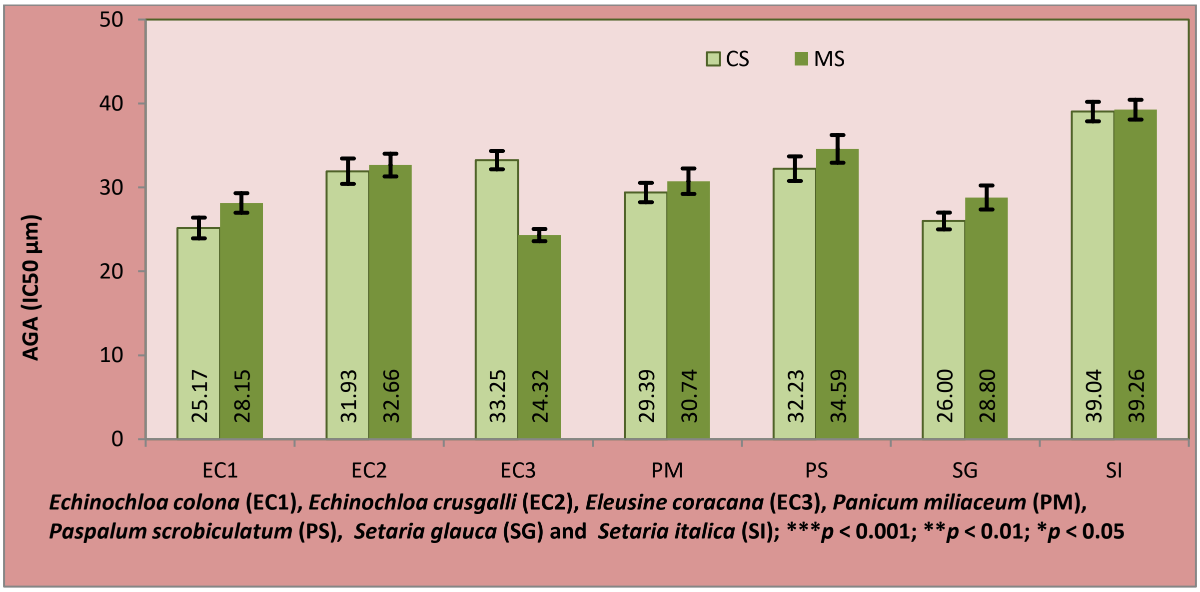


Figure 6: Comparative Anti α-glucosidase activity (AGA) in grains of studied millets in mining site (MS) and control site (CS)

4. CONCLUSION

The highest bioconcentration factor and bioaccumulation was found in *S. glauca* but the translocation efficacy was not at par with that of BCF and BAF. Total sugar content was lower in test samples as compared to control samples and total dietary fiber did not change significantly revealing a positive characteristic of zinc concentrations. The activity of key enzymes i.e. α-amylase and α-glucosidase determine the status of postprandial hyperglycemia. The grains of *E. colona* and *S. glauca* obtained from the mining site revealed the highest

anti α-amylase and anti α-glucosidase activity revealing their preferred usage over other millets in diabetic patients but the zinc concentration in *S. glauca* is more than ingestible permissible limits and hence cannot be subjected for usage as higher levels lead to acute and chronic zinc toxicity causing a disturbance in copper metabolism, reduced iron function and high density lipoproteins, neutropenia, atherogenic cardiac function etc. Higher zinc concentrations also indicate pollution load which may cause bio-magnification if used for prolonged periods. *E. colona*is is safer as it has permissible limits of zinc in grains and is rich in anti α-amylase and anti α-glucosidase activity and therefore can help in better management of glycemic levels in diabetic patients.
Dietary interventions play a preventive and supportive role by protecting against oxidative damage. Such research offers an evidence-based approach and policy suggestions for promoting the consumption of zinc- rich and bioavailable millet varieties as a beneficial nutritional strategy for reducing the risk of Type 2 diabetes Mellitus in the local population.

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