ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

BIOCHEMISTRY

INFLUENCE OF SUGARCANE MONOCROPPING ON RHIZOSPHERE MICROFLORA, SOIL ENZYMES AND NPK STATUS.

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ABSTRACT

Monocropping of sugarcane is highly profitable to the farmers and sugar industry, as it reduces the production cost by 30-40%. But productivity of sugarcane under multiratooning is declining by 30-50% every year due to reduction in cane population per hectare in the state of Maharashtra. The objectives of this study were to explore the changes in beneficial and phytotoxic ratoon cane rhizosphere microflora, soil enzymes and soil NPK level under sugarcane monocropping from high recovery zone and medium recovery zone. The intensive monocropping of sugarcane was found to induce soil sickness due to accumulation of allele chemicals released from sugarcane trash and root exudation, development of phytotoxic microflora and one sided nutrient exhaust. It also caused almost double increase in phytotoxic fungal and bacterial population along with five times more stimulation in the activities of soil enzymes like dehydrogenase, cellulase and amylase. All such alterations correlate with drastic reduction in ratoon cane yield as compared to plant cane.

KEY WORDS

Monocropping, microflora, soil NPK and enzymes, sugarcane

INTRODUCTION

Since ancient times, farmers have recorded the yield reduction in crops under monocropping and sugarcane is also not the exception[1, 2]. The major factors contributing to this phenomenon are the allelochemicals released through decomposition of crop residue and exudation from ratoon cane root system. The perusal of information on monoculturing clearly indicated that soil sickness and sugarcane pest and diseases are the main culprits of monocropping[3, 4, 5].

The problem of soil sickness plays a crucial role in crop ecosystem, all over the world, which is the progressive loss of soil quality due to repeated culture of single crop[6]. This soil loses its potential of crop production, even when sufficient fertilizers and irrigations are applied to the crop. In addition declining of physical and biological properties of soil and negatively influenced population dynamics of beneficial microflora and essential nutrients[7, 8].

Multiratooning of sugarcane is a more sustainable practice giving higher profitable returns in sugarcane growing countries. In India the range of ratooning is usually two to ten[9]. While in the state of Maharashtra, the maximum range of multi-ratooning is only two to four. The correlation between soil enzymatic activities, soil fertility and its microflora is well established[10, 11]. According to Hoagland and Williams[12], microorganisms associated with roots are involved in allelopathic interaction and they trigger release of allelochemicals. The microorganisms modify the root morphology, soil phase's equilibrium and nutrient availability and other chemical soil parameters and also can alter root cells' metabolism and permeability. Processes involved in rhizosphere interactions are complex and dynamic. Both organic and inorganic compounds, nutrients pass through rhizosphere before root absorption. The phytotoxic soil bacteria and fungi produce many toxic secondary metabolites and induce loss in soil fertility under sugarcane monocropping[13]. It has been reported[2] that the impact of soil fatigue and post harvest residues on sugarcane metabolism and yield in prolonged cultivation. The level of changes in all above parameters was compared between the plant cane (control) and 1st, 2nd, 3rd and 4th ratoon of sugarcane cultivars CoC 671 and Co 86032, which are cultivated under ratoon in both recovery zones. Such type of investigation may help to overcome the problem of autotoxicity and reduction in yield in the state of Maharashtra.

MATERIALS AND METHODS

Selected sites

1) Kolhapur region (Adur) was having plant cane considered as control, first ratoon, second ratoon, third ratoon to fifth ratoon and successive cultivation of 1st, 2nd, 3rd and 4th ratoon. This site is characterized as high recovery zone by sugar industry commission Government of Maharashtra. The total cultivation under sugarcane in the state is characterized in high, medium and low recovery zones.
   The soil type in Kolhapur region is red laterite soil with clay particle and pH range 6.42 to 8.10. The average minimum and maximum temperature throughout the year is in between 14°C to 35°C and annual average rainfall is 738 mm.
2) The soil type in Pune region is black cotton soil with 63% clay particles. The pH range in Pune region is in between 6.15 to 7.75 and minimum and maximum temperature throughout the year is in between7°C to39°C and annual rainfall is 722 mm.

Agronomic practices

Agronomic practices for cultivation of plant cane, 1st, 2nd, 3rd and 4th ratoon of sugarcane cultivars CoC 671 and Co 86032 in medium and high recovery zones were recorded. In both the recovery zones chemical fertilizers were not applied through out the cropping season, but there was application of organic manures like FYM, cow dung and neem cake. Flow irrigation was followed at both the sites and the frequency of irrigation was broadly after two weeks. In both the recovery zones and for all the ratoons uniform agronomic practices were followed by cane cultivars.

Collection of soil samples

The rhizosphere soil samples under sugarcane cultivars CoC 671 and Co 86032 were collected at random (at 25 cm depth) from eight months old plant cane, 1st, 2nd, 3rd and 4th ratoon, from high and medium recovery zones. These samples were air dried at room temperature, sieved (2 mm mesh sieve) and stored in vacuum dessicator at 27 ± 2°C.The soil collected from the same regions, which was not under the cultivation of sugarcane, was referred as absolute control and the soil under plant cane was considered as control.

Identification of rhizosphere soil microflora and analysis of microbial count

The soil dilution method was used to identify rhizosphere soil microflora of absolute control (soil not under any type of cane cultivation), plant cane, 1st, 2nd, 3rd and 4th ratoon soil. The rhizosphere soil suspensions were inoculated (1ml) for bacterial cultures on nutrient agar for total plate count[14]. Jensen's medium for nitrogen fixing bacteria, Pikovskaya's medium for phosphate solubilizing bacteria, Czapek's Dox medium for decomposing fungi and MGYP (Malt glucose yeast peptone) medium for actinomycetes were used. 30 g of soil sample was dispensed in 270 ml of sterile distilled water. Following agitation, serial dilutions were set up (up to 10^{-8}), aliquots of 10^{-5} to 10^{-8} concentrations were inoculated in petri plates (90mm) containing sterile media. After uniform mixing, the plates were kept in an incubator at 30 ± 2°C for 3 to 5 days for bacterial growth. For fungal growth the plates were kept in an incubator at 25 ± 2°C for 5 to 10 days. Colony numbers were recorded using a colony counter. The fungi grown on culture medium were identified by using the Dematiaceous Hyphomycetes. Vol. 1[15] , a Manual of the Aspergilli[16] and Manual of the Penicillia[17]. The identification of bacteria was done by Analytical Prophylactic Index Software.

Assay of soil enzymes

Soil dehydrogenase

Activity of this enzyme was assayed by following the method of Casida et al.[18] by reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC). The soil sample (1g) was treated with $CaCO_3$ (50 mg), 3% (w/v) 2, 3, 5-triphenyltetrazolium chloride (1 ml) and the reaction mixture was incubated for 24 h at 37^0C. The triphenyl formazan formed was extracted from the reaction mixture with methanol and the absorbance was recorded at 485 nm in a Shimadzu UV-visible spectrophotometer.

Soil amylase

Above mentioned soil sample (1g) was taken in flask to which 1.5 ml of toluene was added. This mixture was shaken and allowed to stand for 15 min and then 10 ml of distilled water and 5 ml of 2 % (W/V) solution of soluble starch was added. It was kept in an incubator. After five hours the flask was opened and 15 ml distilled water was added. This mixture was centrifuged and one ml of supernatant was used to estimate reducing sugars by modified Nelson-Somogyi method[19]. From these results activity of amylase was calculated.

Soil cellulase

Above mentioned soil sample (5g) was placed in a flask and 0.5 ml of toluene was added. It was mixed thoroughly and after 15 min, 10 ml of acetate buffer (pH 5.9) and 10

ml of 1% carboxymethylcellulose was add into this reaction mixture. The flask was then incubated for 24 hours at 30^0C. At the end 50 ml distilled water was added and the final volume was made up to 100 ml. The reducing sugars produced as a result of an activity of this enzyme were determined[19].

Analysis of soil NPK contents

The above mentioned soil sample (100g) was used for the analysis of NPK content by using the standard methods[3, 20].

Statistical analysis

The data were summarized as the pooled means of five replicates, collected over two years (2005-2006 and 2006-2007), with standard deviation as a measure of variability. One way ANOVA was used to compare the different samples. Fisher's LSD was applied as a post hoc test at $P<0.05$. All calculations were performed with Sigma stat (release 3.5) and Microsoft Excel (Office 2000).

RESULTS AND DISCUSSION

Rhizosphere soil microflora identification

Micro environment and microflora in contact with plant roots can be altered by exudates containing different types of organic and inorganic compounds. Some exudates metabolites stimulate the microbial growth, while others inhibit it. The nature of root exudates determines microbial balance in rhizosphere soil, which may have direct or indirect effects on plant growth and development of recipient plant[11, 12]. The released allelochemicals from leachates, residues and root exudates can play a significant positive or negative role (s).

The data on rhizosphere soil microflora indicated that different types of harmful fungi and bacteria developed in rhizosphere soil of sugarcane under monocropping as compared to absolute control and plant cane of both cultivars in medium recovery zone (Table 1). With increasing number of multi-ratooning the occurrence of harmful fungi like *Fusarium oxysporum, F. moniliformae, Rhizoctonia, Cladosporium, Alternaria, Aspergillus niger* was becoming more frequent. Similarly the harmful bacteria like *Enterobacter* and *Agrobacter* were detected frequently under monocropping. It has been suggested that[21, 22] the allelochemicals like tannins and phenols, chlorogenic, elagic, ferulic and *p*-coumaric acids interfere with soil microbial activities and their population. They further explained that such allelochemicals affect relative concentration of soil ammonia and nitrates as well as nitrogen fixation and nitrification.

Some research workers[23] have claimed that microorganisms affect plant productivity through their impact on soil physical and chemical properties as well as availability of nutrients. It is also reported[24, 25] that the autotoxicity and soil fatigue as well as loss in fertility are created under monocropping of sugarcane. They attributed these changes in soil to enhanced population of phytotoxic fungi and bacteria as well as to the secondary metabolites secreted by them. Amongst microbial species, *Fusarium oxysporum* was the most dreadful, because it secretes fusaric acid, which is mainly responsible for autotoxicity in ratoon sugarcane[26]. The findings of present study were supported by many researchers[24, 27, 28]. They reported *Aspergillus, Fusarium* and *Cladosporium* species from rhizosphere soil of sugarcane ratoon and claimed that the autotoxicity was due to secondary metabolites secreted by them, leading to yield reduction.

Other studies have reported that pathogenic fungi were associated with loss in soil fertility and microbial population under sugarcane monocropping, due to accumulation of different types of allelochemicals[29, 30, 31], by correlating rhizosphere fungi with growth and yield of sugarcane. The production of phytotoxins by these fungi was responsible for poor sugarcane growth in Taiwan[1, 32]. With increasing frequency of ratoon (i.e. 2nd ratoon

onwards) there was increase in different types of fungi, such as *Aspergillus flavus, Penicillium, Aspergillus candidus, Rhizoctonia, Curvularia, Cladosporium cladosporioides, Fusarium oxysporum* etc. and bacteria and actinomycetes. This increase can be attributed to the allelochemicals released from trash and old roots of ratoon sugarcane plants , along with other soil factors. According to Sampietro[24] yield decline in sugarcane was associated with soil borne problems such as increase in population of phytoxic fungi, loss in benficial microflora, loss or exaust of nutiernts, increase in soil pH and changes in soil physico-chemical properties. The results of present study on above mentioned aspects also corroborate with the above findings.

Table 1
Identity of rhizosphere microbial isolates obtained from sugarcane cultivars CoC 671 and Co 86032 from medium recovery zone under monoculturing.

Frequency of ratooning	Fungal species		Bacteria and Actinomycetes	
	CoC 671	Co 86032	CoC 671	Co 86032
*Control	*Aspergillus flavus, Penicillium expansum,*	*Aspergillus flavus, Penicilliumexpansum,*	Actinomycetes	*Agrobacter radiobacter*
Plant cane	*Aspergillus flavus , Penicillium expansum, Alternaria alternata*	*Aspergillus flavus, Penicillium expansum,*	*Staphylococcus lentus*	*Enterobacter erogens*
1st	*Aspergillus flavus, Penicillium expansum, Fusarium oxysporum, Paecilomyces marquandi*	*Aspergillus niger, Penicillium expansum, Fusarium oxysporum, Alternaria alternata*	*Agrobacter radiobacter, Enterobacter gergoviae*	*Agrobacter radiobacter*
2nd	*Aspergillus niger, Penicillium expansum, Fusarium oxysporum*	*Aspergillus flavus , Penicillium expansum, Fusarium moniliforme, Verticillium albo-atrum*	Actinomycetes	*Agrobacter radiobacter*
3rd	*Aspergillus flavus, Fusarium moniliforme, Rhizoctonia , Curvularia lunata, Cladosporium cladosporioides, Trichoderma viride*	*Aspergillus flavus, Penicillium expansum, Fusarium oxysporum, Alternaria alternata, Verticillium albo-atrum*	*Brevundimonas diminuta, Azotobacter*	*Azotobacter*
4th	*Aspergillus flavus, Penicillium expansum, Paecilomyces marquandi, Trichoderma viride*	*Aspergillus niger, Penicillium expansum, Curvularia lunata, Cladosporium cladosporioides*	*Echerichia coli*	Actinomycetes

**Control- Absolute control: soil not under any type of cane cultivation.*

• The data on microflora recorded is based on three times observations of the rhizosphere soil analysis.

Plant cane: First crop, 2nd, 3rd, 5th and 6th represents number of years of continuous cropping of sugarcane (ratooning frequency).

Rhizosphere soil microbial counts

Rhizosphere soil *Azotobacter* counts under monocropping were increased significantly with frequency of ratooning as compared to control in medium recovery zone under both cultivars (Table 2). These changes were considered as sensitive indicators of soil quality[22]. The changes in microbial counts are usually in response to organic matter cycles within the soil[33, 34]. The latter authors claimed that microbial biomass fluctuates due to changes in available carbon substrates and nutrients. After harvesting at the end of ratoon crop there is amendment or incorporation of 5 to 6 tons of crop residue per hectare. This crop residue (sugarcane dried leaves) is adding organic carbon in the soil and might be responsible to induce the changes in soil microflora. This may be the reason for variations of microbial counts under monocropping of sugarcane. There was no correlation between frequency of ratooning and phosphate solubilizing bacteria, residue decomposing and other microbes.

Table 2

Rhizosphere soil microbial counts (CFUs /g soil dry weight) for sugarcane cultivars CoC 671 and Co 86032 from medium recovery zone under monoculturing.

Frequency of ratooning	Azotobacter		Phosphate solubilizing bacteria		Residue decomposing microbes		Other microbes	
	CoC 671	Co 86032	CoC 671	Co 86032	CoC 671	Co 86032	CoC 671	Co 86032
*Control	$02x10^4$	$01x10^4$	$05x10^7$	$02 x10^7$	$02x10^7$	$03x10^3$	$12x10^7$	$10x10^7$
Plant cane	$03x10^4$	$01x10^4$	$06x10^7$	$02 x10^7$	$03x10^7$	$05x10^3$	$14x10^7$	$11x10^7$
1st	$02x10^5$	$02x10^5$	$07x10^7$	$05x10^7$	$04x10^7$	$01x10^7$	$17x10^7$	$12x10^7$
2nd	$02x10^5$	$02x10^5$	$09x10^7$	$06x10^7$	$05x10^5$	$03x10^3$	$17x10^7$	$12x10^7$
3rd	$03x10^5$	$03x10^6$	$06x10^7$	$2.5x10^8$	$07x10^7$	$03x10^7$	$20x10^7$	$06x10^7$
4th	$03x10^7$	$03x10^7$	$05x10^7$	$06x10^7$	$01x10^7$	$03x10^7$	$12x10^7$	$20X10^8$
LSD 0.05	$3.63x10^6$	$3.65x10^6$	$1.62x10^7$	$1.78x10^7$	$5.69x10^6$	$3.29x10^6$	$2.62x10^7$	$1.46x10^8$
Significance	**	**	**	**	**	**	**	**

CFUs - Colony Forming Units

**Control- Absolute control: soil not under any type of cane cultivation.*

Plant cane: First crop, 2nd, 3rd, 5th and 6th represents number of years of continuous cropping of sugarcane (ratooning frequency).

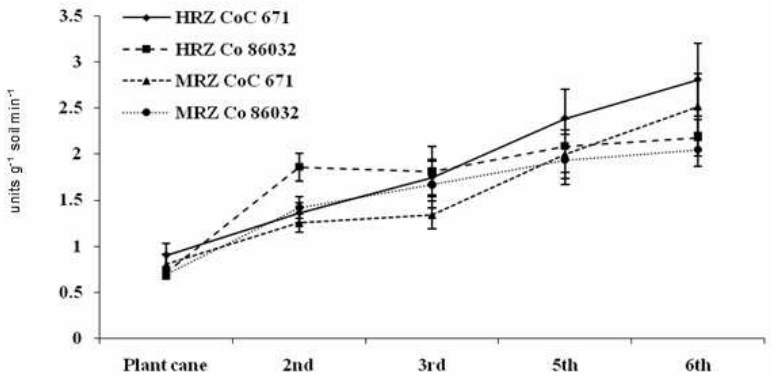
Soil enzyme activity

In high and medium recovery zones, dehydrogenase, cellulase and amylase

activities in soil under both cultivars were increased significantly due to monocropping (Figs 1-3). The stimulation was recorded[35] in the activities of different soil enzymes with continuous cultivation of sugarcane, cassava and pineapple. The involvement of dehydrogenase activity was indicated[22, 33] in the breakdown of soil organic matter and microbial activity. During monocropping of sugarcane, after every successive harvesting of ratoon crop, huge biomass of sugarcane trash (dried cane leaves) is added into the soil, which might be responsible for stimulating the activities of above mentioned soil enzymes. It was claimed by many workers that [36, 37, 38] enhanced activities of such enzymes were due to addition of organic matter in the soil, which promoted microbial activities. Decomposition of crop residues contributes to autotoxic effect observed for several crop species like rice, asparagus, alpha-alpha and sugarcane. One of the best examples demonstrating auto, intoxication mechanism mediated by decomposing harvest residue is for rice[39]. He observed yield reduction in second annual rice crop by 25% in Taiwan. Amount of phytotoxins produced by rice residue was dependent by environmental conditions such as humidity, temperature, soil minerals etc. The composition of organic matter determines the soil properties (nutrient availability, microbial population and soil fauna) which will affect the germination and growth of plants. Apart from this, accumulation of different allelochemicals with increasing concentrations along with increasing frequency of ratooning might be the additional cause for inducing stimulation in activities of above mentioned soil enzymes.

Fig. 1

Effect of monocropping on activity of dehydrogenase in soil under sugarcane cultivars CoC 671 and Co 86032 from high and medium recovery zones (HRZ and MRZ, respectively).



	HRZ CoC 671	HRZ Co 86032	MRZ CoC 671	MRZ Co 86032
LSD $_{0.05}$	0.33	0.28	0.29	0.26
Significance	**	**	**	**

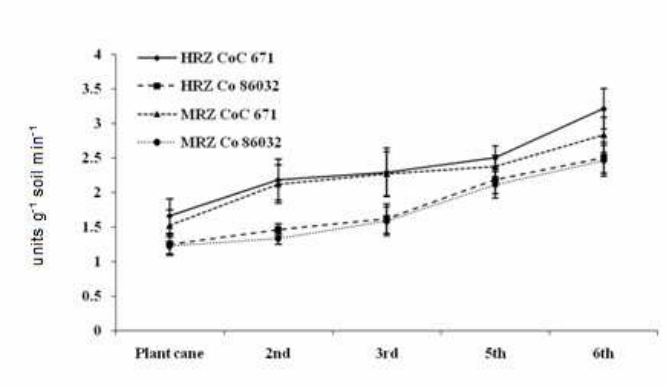
[#] *Data are mean values (n=5) with error bars as ±standard deviation. '**' represent significance at p<0.01.*

Fisher's LSD was applied as a post hoc test at p<0.05.

Plant cane: First crop, 2nd, 3rd, 5th and 6th represents number of years of continuous cropping of sugarcane (ratooning frequency).

Fig. 2

Effect of monocropping on activity of cellulase in soil under sugarcane cultivars CoC 671 and Co 86032 from high and medium recovery zones (HRZ and MRZ, respectively).



	HRZ CoC 671	HRZ Co 86032	MRZ CoC 671	MRZ Co 86032
LSD $_{0.05}$	0.38	0.24	0.34	0.23
Significance	**	**	**	**

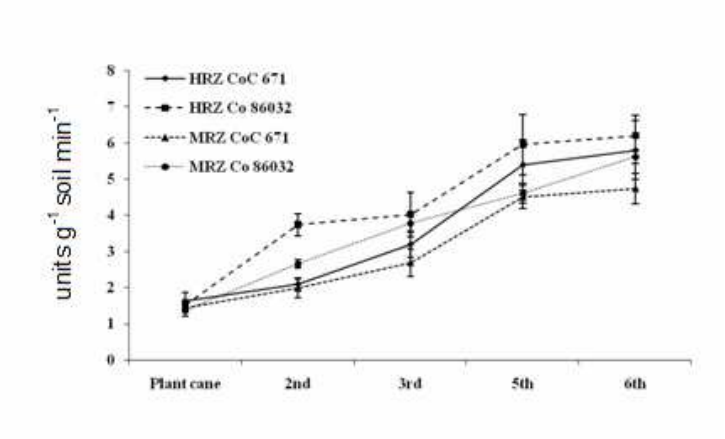
*# Data are mean values (n=5) with error bars as ±standard deviation. '**' represent significance at p<0.01.*

Fisher's LSD was applied as a post hoc test at p<0.05.

Plant cane: First crop, 2nd, 3rd, 5th and 6th represents number of years of continuous cropping of

Fig. 3

Effect of monocropping on activity of amylase in soil under sugarcane cultivars CoC 671 and Co 86032 from high and medium recovery zones (HRZ and MRZ, respectively).



	HRZ CoC 671	HRZ Co 86032	MRZ CoC 671	MRZ Co 86032
LSD $_{0.05}$	0.69	0.72	0.43	0.50
Significance	**	**	**	**

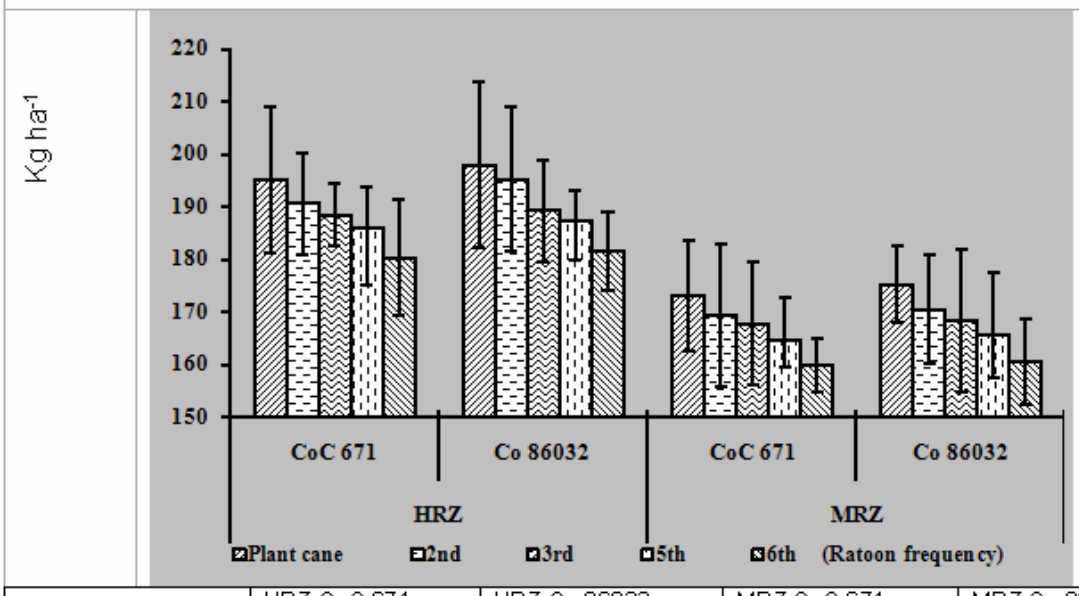
*# Data are mean values (n=5) with error bars as ±standard deviation. '**' represent significance at p<0.01.*
Fisher's LSD was applied as a post hoc test at p<0.05.
Plant cane: First crop, 2nd, 3rd, 5th and 6th represents number of years of continuous cropping of sugarcane (ratooning frequency).

Rhizosphere soil NPK contents

The rhizosphere soil N, P and K levels under monocropping were negatively influenced with increasing frequency of ratooning in medium and high recovery zones, but the results are non significant (Figs 4-6). This reduction in NPK level might be due to disturbance in the relationship between the root system of cane plant and rhizosphere soil. The one sided soil exhaustion of NPK during monoculturing has been reported[26]. NPK levels were found to be correlated with yield potential in ratoon[40, 41]. Few researchers were of the opinion that[42, 43] maintaining adequate levels of macro and micronutrients were essential to obtain higher cane yields. Allelochemicals can interfere with nitrogen, phosphorus, potassium, magnesium, calcium and iron uptake[44, 45, 46, 47].

Fig. 4

Effect of monocropping on nitrogen content in soil under sugarcane cultivars CoC 671 and Co 86032 from high and medium recovery zones (HRZ and MRZ, respectively).



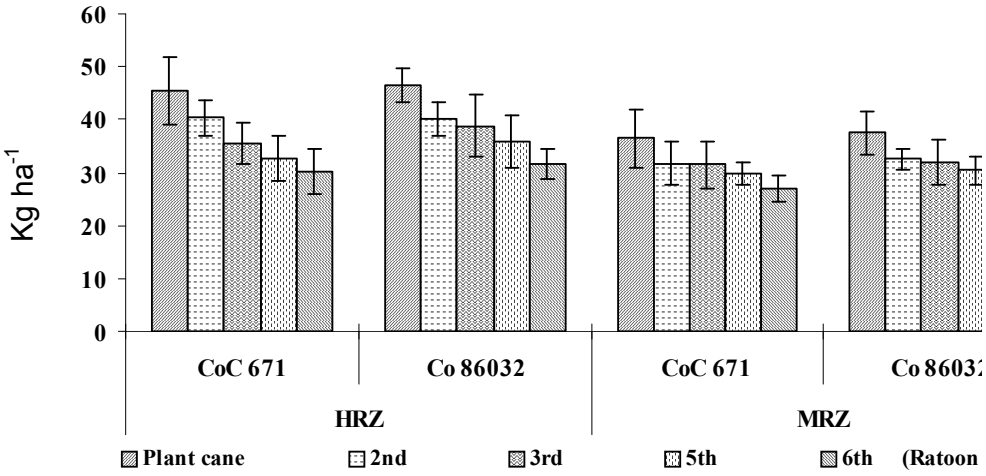
	HRZ CoC 671	HRZ Co 86032	MRZ CoC 671	MRZ Co 86032
LSD $_{0.05}$	-	-	-	-
Significance	ns	ns	ns	ns

Column data are mean values (n=5) with error bars as ±standard deviation. 'ns' represent non-significance. Fisher's LSD was applied as a post hoc test at $p<0.05$.

Plant cane: First crop, 2nd, 3rd, 5th and 6th represents number of years of continuous cropping of sugarcane (ratooning frequency).

Fig. 5

Effect of monocropping on phosphorus content in soil under sugarcane cultivars CoC 671 and Co 86032 from high and medium recovery zones (HRZ and MRZ, respectively).

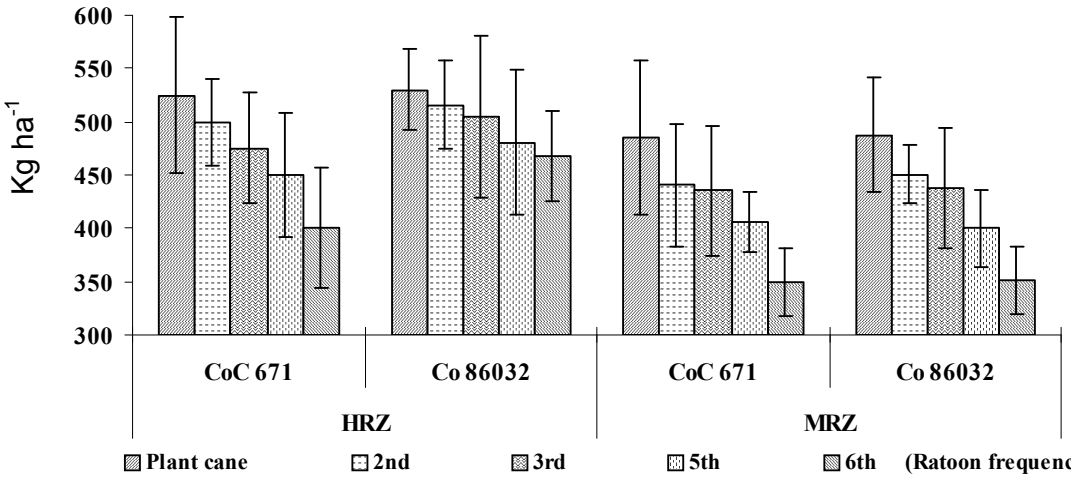


	HRZ CoC 671	HRZ Co 86032	MRZ CoC 671	MRZ Co 86032
LSD $_{0.05}$	5.99	5.56	5.18	4.27
Significance	**	**	*	**

Column data are mean values (n=5) with error bars as ±standard deviation. '', '**' represent significance at p<0.05, p<0.01 respectively.*
Fisher's LSD was applied as a post hoc test at p<0.05.
Plant cane: First crop, 2nd, 3rd, 5th and 6th represents number of years of continuous cropping of sugarcane (ratooning frequency).

Fig. 6

Effect of monocropping on potassium content in soil under sugarcane cultivars CoC 671 and Co 86032 from high and medium recovery zones (HRZ and MRZ, respectively).



	HRZ CoC 671	HRZ Co 86032	MRZ CoC 671	MRZ Co 86032
LSD $_{0.05}$	75.61	-	70.25	56.67
Significance	*	ns	*	**

Column data are mean values (n=5) with error bars as ±standard deviation. '', '**' and 'ns' represent significance at p<0.05, p<0.01 and non-significance, respectively.*
Fisher's LSD was applied as a post hoc test at p<0.05.

Plant cane: First crop, 2nd, 3rd, 5th and 6th represents number of years of continuous cropping of sugarcane (ratooning frequency).

Effect of monoculturing on yield parameters of sugarcane ratoon

Number of millable canes/stool

The results recorded in table (Table 3) had indicated drastic reduction in no. of millable canes per stool in both the cultivars. The degree of reduction was increased with the increasing frequency of ratooning. The range of reduction observed was from 12.00 and 13.00 % (plant cane) to 6.00 to 7.15 % (4th ratoon).

Weight of millable cane

Considerable reduction in weight of millable cane was noted in CoC 671 and Co 86032 as compared to plant cane. The level of reduction was increased from plant cane (2.15 and 2.25 kg) to sixth ratoon, which showed the highest reduction (1.05 and 1.10 kg).

It was showed that[48] number of millable canes per stool, single cane weight and stalk diameter reflected highly positive direct effect on ratoon yield at genotypic level. The single cane weight, cane diameter and no. of tillers per stool are also the important characters contributing towards cane yield[49]. The increased potassium causing profuse tillering in sugarcane was also observed[50].

Table 3
Effect of monoculturing on yield parameters of sugarcane cultivars CoC 671 and Co 86032 from high recovery zone.

Frequency of ratooning	No. of millable canes/stool		Weight of millable cane (kg)	
	CoC 671	Co 86032	CoC 671	Co 86032
Plant cane	12.00 ±1.68	13.00 ±0.91	2.15 ±0.32	2.25 ±0.24
1st	10.10 ±0.81	11.20 ±0.90	1.95 ±0.25	2.00 ±0.12
2nd	8.50 ±0.93	9.35 ±1.40	1.35 ±0.18	1.50 ±0.19
3rd	6.30 ±0.82	8.50 ±1.19	1.18 ±0.08	1.20 ±0.10
4th	6.00 ±0.84	7.15 ±0.64	1.05 ±0.09	1.10 ±0.09
LSD $_{0.05}$	1.42	1.38	0.28	0.22
Significance	**	**	**	**

 # Data are mean values (n=5) followed by ±standard deviation. '', '**' and 'ns' represent significance at p<0.05, p<0.01 and non-significance, respectively.*

Table 4
Effect of monoculturing on yield parameters of sugarcane cultivars CoC 671 and Co 86032 from medium recovery zone.

Frequency of ratooning	No. of millable canes/stool		Weight of millable cane (kg)	
	CoC 671	Co 86032	CoC 671	Co 86032
Plant cane	14.00 ±1.96	16.25 ±1.14	2.00 ±0.30	2.15 ±0.23
1st	11.50 ±0.92	14.50 ±1.16	1.75 ±0.22	1.85 ±0.11
2nd	9.50 ±1.04	10.00 ±1.50	1.45 ±0.20	1.50 ±0.19
3rd	9.12 ±1.18	9.75 ±1.36	1.50 ±0.10	0.90 ±0.08
4th	8.15 ±1.14	8.95 ±0.81	1.10 ±0.099	1.15 ±0.10
LSD $_{0.05}$	1.73	1.61	0.27	0.21
Significance	**	**	**	**

 # Data are mean values (n=5) followed by ±standard deviation. '', '**' and 'ns' represent significance at p<0.05, p<0.01 and non-significance, respectively.*

Chemical interference by allelochemicals plays an important role in crop yield considering monocropping. The productivity of agricultural crops can be affected by allelochemicals released from crops, direct and indirect action on microorganisms, available nutrients was also indicated[51]. In sugarcane yield reduction under monocropping can be correlated with development of phytotoxic microflora, loss in major soil nutrients like NPK and changes in the activities in the soil enzymes.

In conclusion, our findings indicated a significant decrease in NPK contents during sugarcane monocropping. It remains to be assessed whether such a loss of soil fertility could be linked to the observed increases in microbial diversity and colony numbers. The stimulated activities of soil dehydrogenase, cellulase and amylase most likely correlated with amendment of organic matter in ratoon soil in the form of sugarcane trash (5 to 6 Tons/ ha).

ACKNOWLEDGEMENTS

First author is grateful for the financial support in terms of teacher fellowship from University Grants Commission, New Delhi. Authors also acknowledge Head, Department of Botany, University of Pune, for providing the research facilities.

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