

Effect of Metal Contamination and Pesticides on the Cellulase-producing Microbes Present in the Gut of *Perionyx Excavatus*

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Abstract

Cellulase-producing microorganisms were isolated and identified as *Micrococcus luteus*, *Bacillus megaterium* and *Enterobacter cloacae* among the bacteria and *P. boydii*, *Streptomyces sp.* and *Candida sp.* among the fungal isolates from the gut of *Perionyx excavatus*. They were then subjected to two different doses of metal Zinc ((Dose I- 60mg/Kg and Dose II- 120 mg/Kg.) and two doses of two commonly used pesticides (Pendimethalin and Pretilachlor). It has been observed that out of the six cellulase-producing microbes, the higher dose of the metal was proved to be detrimental for three of them. *Bacillus megaterium*, that was found to be producing a sufficient amount of the enzyme, was absent even when it has been exposed to the lower dose of the metal. Also, the fungal isolates showed a decrease in number when exposed to higher dose of the contaminants. But the cellulase-producing bacterial isolates like *Micrococcus luteus* and *Enterobacter cloacae* were not at all affected by the presence of either metal or the pesticides. The experiment throws light on the microbe-earthworm relationship and effect of toxins and metals on the commercially- important microbes present in the soil and earthworm's gut. The isolates that were present even at the higher doses of the metal and pesticides would be very promising for the production of large amount of the enzyme cellulase.

1. Introduction

Cellulose is considered as the most abundant biopolymer on earth. The extensive potential of cellulose as a renewable source of energy was recognized only after the discovery of cellulases (Bhat and Bhat, 1997). Many industries implicate cellulases on a regular basis (Hoshino et al., 1997; Lynd et al., 2002). Several microorganisms are capable of producing cellulases that act in a synergistic manner to hydrolyse the β -1, 4-D glucosidic bonds within the cellulose molecules (Akiba et al., 1995). The cellulosic enzyme system consists of three major components, i.e., endoglucanases, exoglucanases and β - glucosidases (Parry et al., 1983; Gielkenes *et al.*, 1999; Kang et al., 1999). Cellulase is produced by a large number of microorganisms. They can either be cell bound or extracellular in nature. However, a large number of microbes can be cellulose-degrading, but only a few of them can produce significant amounts of free enzymes capable of completely hydrolyse crystalline cellulose (Koomnok, 2005).

Nowadays huge amount of agricultural, industrial and household cellulosic wastes have been accumulating in environment. It has now become an economic interest to develop an effective and reliable method to hydrolyze this cellulosic biomass. During their growth on Cellulosic matters, microorganisms produce these inducible bioactive compounds called cellulases (Lee and Koo, 2001). A number of investigations have reported the degradation of cellulosic materials by cellulases, but only few studies have examined the microorganisms that had met the industrial requirement. Fungi are the main cellulase-producing microorganisms as only a few bacteria have been reported to produce cellulase activity. One of the industrial applications of cellulases includes the "Biopolishing" of fabrics for increasing its softness and brightness in textile industries. Animal feeds were incorporated with cellulases for improving the nutritional quality and digestibility. De-inking of paper is another emerging application of these cellulases. It plays a vital role in the conversion of cellulosic biomass into

commodity chemicals (Gong et al., 1999; Himmel et al., 1999). Lee and Koo (2001) showed that production of cellulase was the most expensive step during ethanol production from the cellulosic biomass where it accounted for almost 40% of the total cost. To obtain cheap ethanol, successful screening of novel cellulase producing strains is very important. Industrial bioconversions of lignocelluloses requires multifunctional cellulases with broader substrate utilization, also, the enzymes can work efficiently in a wide range of pH and temperature conditions used in the bioconversion of cellulosic material to bioethanol.

Cellulose is one of the most important source of carbon on the plant. Cellulose degradation and its subsequent utilization is important for global carbon source. It is very important to understand factors that control cellulase production. Cellulase biosynthesis in many organisms appears to be regulated by availability of readily metabolizable carbon such as glucose. This readily metabolizable carbon represses the synthesis of enzymes related to catabolism of alternate carbon source including cellulose.

Microorganisms have been highly exploited for their abilities to produce a wide variety of cellulases. Mostly, the emphasis has been placed on the use of fungi as they produce substantial amounts of cellulases and hemicellulases which are secreted into the medium for easy extraction and purification. Culturable, cellulase-producing bacteria have been isolated over the years from a wide variety of sources such as composts, decaying plant materials from forest or agricultural waste, the faeces of ruminants, soil and organic matter, and extreme environmental conditions like hot-springs, to name a few (Doi, 2008). Such microbes have also been known to present as the gut microflora of many earthworm.

Earthworms accelerate microbial activities within the soil by increasing the population of soil microorganisms (Binet *et al.*, 1998). The ability of earthworms to increase plant nutrient availability might depend on the activity of microflora present in earthworm gut. These microbes mineralize the organic matter and also facilitate the chelation of metal ions (Pizl and Novokova 1993; Canellas et al; 2002). But in contrast the metals are causing an adverse effect on the earthworm gut microflora which may also lead to reduced number of cellulase-producing microbes in earthworm gut and cast.

Earthworms are also found in soils containing high levels of metals (Spurgeon and Hopkin 1996; Langdon et al., 2001; Vijver et al., 2007) and these earthworms constitute a major part of soil fauna. Earthworms, generally, increase the mobility and availability of certain metals and metalloids in the soil (Sizmur and Hodson, 2009) and this may result in higher concentrations of these metals leaching out of the soil into ground water (Tomlin et al., 1993). They may also reduce the efficiency of soil remediation by mobilizing recalcitrant metals (Udovic *et al.*, 2007). The mechanism for this phenomenon is though unclear, but it may involve changes in the microbial populations, pH, dissolved organic carbon or metal speciation (Sizmur and Hodson, 2009).

Metal toxicity in soils are determined by the bioavailable metal rather than total metal concentrations (Harmsen, 2007) and this depends on mobility and speciation of metal in the living soil environment (Di Toro et al., 2001; Thakali et al., 2006; Arnold *et al.*, 2007). Earthworms are able to survive and reproduce in anthropogenically metal-contaminated soil (Spurgeon et al., 1994) and while going through this process,

they may also accumulate high concentration of metals within their bodies (Hobbelen *et al.*, 2006). There are many studies that have reported the detrimental impact of metals in soil and also on the inhabiting earthworms and their gut-associated microflora (Nahmani *et al.*, 2007). Several studies have demonstrated that earthworm gut contains aerobic microorganisms in abundance (Dash *et al.*, 1986; Karsten and Drake, 1995). Some aerobes have been shown to even proliferate during its passage through the earthworm's gut and they reach densities greater than those in the soil (Fischer *et al.*, 2007; Kistuek *et al.*, 2007; Parthasarathi *et al.*, 2007). Earthworms speed up soil reclamation and make them productive by restoring beneficial microflora within their gut (Nakamura, 1996).

Human activities produce approximately 38 billion metric tons of organic waste each year worldwide (Prasad, 2011). The use of pesticides in agricultural practices often results in loss of biodiversity (Hole *et al.*, 2005). Earthworms are known as ecosystem engineers (Jones *et al.*, 1994), and they influence the dynamics of organic matter in the soil. Generally, ecotoxicological studies on earthworms have focused on metals but the effect of pesticides has been studied less (Lowe and Butt, 2007). Though some studies showed that older pesticides show greater inhibitory effects on earthworms than the newer ones (Tu *et al.*, 2011). Earthworms' activity can be ceased by the activity of pesticides. Globally, earthworms are used as biomarkers for evaluating chemical environment pollution (Bostos-Oberg and Goicochea, 2002). The significance of earthworms has been well acknowledged but yet it requires sufficient data on the toxicity of specific pesticides to such non-target organisms in order to select chemicals that induce less harm to them. Most pesticides have a detrimental effect on several species of earthworms. Several studies have assessed the toxic impacts of pesticides on earthworms (Thompson, 1971; Randall, Butler and Hughes, 1972; stenersan, 1979; Inglesfied, 1984; Roberts and Dorough, 1985; and Potter *et al.*, 1990).

Since cellulase has been reported as one of the commercialized and economically important products for the bioconversion of cellulosic materials, the present study has been taken up to investigate the effect of two commercially used pesticides and metal zinc on cellulase-producing microbes isolated from the gut of earthworm, *Perionyx excavatus*.

2. Materials And Methods

2.1. Period of Study

The period of study was pre monsoon and post monsoon season of 2014 and 2015.

2.2. Collection and extraction of test specimen

Specimens of *Perionyx excavatus* were collected from the grasslands around Midnapore town (West Bengal, India), that has never been used for agricultural purpose and pest control. The specimens were brought to the laboratory and were cultured in large earthen pots. Finely ground soil which has been collected from the same grassland and farmyard manure mixed in the ratio of 1:1 was used as culture medium. The rest of the conditions were kept standard as described by Samanta and Das, 2016.

2.3. Pesticides Used

Commercially available pesticides were used in the present study. Technical information of these pesticides is given in Table 1.

Table 1
The insecticides used in the present study along with their commercial name and recommended agricultural doses

Pesticide name	Trade name	Formulation	Manufacturer	RAD* (mg/Kg)
Pendimethalin	Kristop	30 EC	Krishi Rasayan Exports Pvt. Ltd., Samba, J&K.	500
Pretilachlor	Prince	50 EC	Krishi Rasayan Exports Pvt. Ltd., Samba, J&K.	500
*RAD- Recommended Agricultural Dose				

2.4. Test metal and doses

The metal we used for this experiment is Zinc (Zn). The pollutant was added in form of ZnSO₄. The earthworms were exposed to two doses of this metal i.e. Dose I- 60mg/Kg and Dose II- 120mg/Kg.

2.5. Method of exposure to the metal

Thirty clitellate adult *Perionyx excavatus* weighing 300-600mg in three replicates exposure chamber, containing 1 Kg dry mass of artificial soil and 4 Kg of feed material were chosen for the experiment. The two doses (Dose I and Dose II) of metal were then added to the dry soil and mixed well. The moisture content was maintained for 60% at 28 ± 2°C and 12L/12D photoperiod. The feed was not changed during the experiment period. The earthworms were then exposed separately to the respective chambers for a period of 7 days. A control set was also maintained without the metal.

2.6. Method of exposure to pesticides

Two dilutions of the pesticide formulations based on their recommended agricultural doses as provided by the manufacturer (viz. ½ RAD, ¼ RAD) were used to determine the LC50 of the selected pesticides on *Perionyx excavatus*.

Then to study the effects of sub-lethal doses of the pesticide formulations, doses based on their respective LC50 values (i.e. ½ LC50 and ¼ LC50) were applied as described by Sanyal et al., 2015.

The doses for the exposure for both the pesticides and metal has been summarized in Table 2.

Table 2

Table showing the Exposure Dose and Time period for both the pesticides and metal for *Perionyx excavatus*.

Exposed to	Days of Exposure
Pesticide 1 Pendimethalin (LC50-0.16mg/Kg)	3 Days
Dose I-¼LC50 Dose II-½ LC50	
Pesticide 2 Pretilachlor (LC50-0.052mg/Kg)	3 Days
Dose I-¼LC50 Dose II-½ LC50	
Metal Zinc	7 Days
Dose I- 60mg/Kg Dose II- 120mg/Kg	

2.7. Isolation and characterization of gut microflora of *Perionyx excavatus*:

The isolation and characterization of the gut microbes of *Perionyx excavatus* was done as method described by Samanta and Das, 2016. Also, the probable nomenclature of the isolated earthworm gut microflora has been identified (Samanta and Das, 2016).

2.8. Cellulase Assay

Further, the bacterial and fungal isolates were then inoculated on Carboxy Methyl Cellulose (CMC) agar to screen them for cellulose activity. Cellulose-degrading microbes would be selected based on the formation of sufficient clearing zones on CMC agar.

2.9. Quantitative measurement of cellulase production by the microbes:

Cellulase activity was measured using filter paper as substrates according to the DNS method (Ghose, 1987).

2.10. Effect of Zinc metal and the pesticides on isolated gut microflora

The CFU of the isolated microflora was calculated for the control and the two doses of metal and both the pesticides to see if the metal and pesticide exposure had any detrimental effect on them.

3. Results

1. Nomenclature of the microflora isolated:

Based on the observations made in the biochemical tests, the probable nomenclature (Table 3) of the isolated earthworm gut microflora has been done (Samanta and Das, 2016).

Table 3
Nomenclature of the isolated bacterial
and fungal isolates

Isolates	Probable nomenclature
B1	<i>Bacillus cereus</i>
B2	<i>Enterococcus faecalis</i>
B3	<i>Micrococcus luteus</i>
B4	<i>Bacillus megaterium</i>
B5	<i>Enterobacter cloacae</i>
F1	<i>Aspergillus fumigates</i>
F2	<i>Pseudallescheria boydii</i>
F3	<i>Streptomyces sp.</i>
F4	<i>Nocardia sp.</i>
F5	<i>Candida sp.</i>

3.2. Screening of Cellulase activity:

Out of the isolated bacterial and fungal isolates, few showed a positive cellulase activity (Fig. 1; Table 4).

Table 4
Table showing the results of the Cellulase activity test of the isolates

Isolates	Cellulase activity
<i>Bacillus cereus</i>	Negative
<i>Enterococcus faecalis</i>	Negative
<i>Micrococcus luteus</i>	Positive
<i>Bacillus megaterium</i>	Positive
<i>Enterobacter cloacae</i>	Positive
<i>Aspergillus fumigatus</i>	Negative
<i>Pseudallescheria boydii</i>	Positive
<i>Streptomyces sp.</i>	Positive
<i>Nocardia sp.</i>	Negative
<i>Candida sp.</i>	Positive

3.3. Measurement of cellulase production by the microbes-

The amount of cellulase production in 48 hours by each of the cellulase-producing microflora was then compared (Fig. 2).

3.4. Effect of metal exposure-

No mortality was observed among any treated group of earthworms. But the number of gut microflora varied in different exposures which have been summarized below in Table 5.

Table 5
Summarized effects of both the doses of metal on the isolates.

Isolates	Abundance/Presence		
	Control	MDI	MDII
<i>Bacillus cereus</i>	++++	+++	
<i>Enterococcus faecalis</i>	+++	++	
<i>Micrococcus luteus</i> (Cellulase-producing)	++++	+	++
<i>Bacillus megaterium</i> (Cellulase-producing)	++++		
<i>Enterobacter cloacae</i> (Cellulase-producing)	++++	+	+++
<i>Aspergillus fumigatus</i>	++++	+++	++
<i>Pseudallescheria boydii</i> (Cellulase-producing)	+++	++	+
<i>Streptomyces sp.</i> (Cellulase-producing)	+++	+	+
<i>Nocardia sp.</i>	++	++	+
<i>Candida sp.</i> (Cellulase-producing)	+++	++	

+ Presence, ++ Average growth, +++/more Abundance, - Absence

For further confirmation of the effect of metal doses on the isolated microflora, CFU was calculated for each of the isolate and compared with the control (Table 6).

The data obtained from the cfu calculation for each strain were then compared with the control (Fig. 3)

Table 6
CFU calculation of each isolate

Isolates	CFU/ml		
	Control	Metal Dose I	Metal Dose II
<i>Bacillus cereus</i>	243X 10 ⁵	129X10 ⁵	17X10 ⁴
<i>Enterococcus faecalis</i>	423X10 ⁵	194X10 ⁵	65X10 ⁴
<i>Micrococcus luteus</i> (Cellulase-producing)	156X10 ⁵	59X10 ⁵	62X10 ⁵
<i>Bacillus megaterium</i> (Cellulase-producing)	273X10 ⁴		
<i>Enterobacter cloacae</i> (Cellulase-producing)	194X10 ⁴	114X10 ⁴	171X10 ⁴
<i>Aspergillus fumigatus</i>	23X10 ³	09X10 ³	
<i>Pseudallescheria boydii</i> (Cellulase-producing)	52X10 ²	31X10 ²	02X10 ²
<i>Streptomyces sp.</i> (Cellulase-producing)	16X10 ³	2X10 ³	
<i>Nocardia sp.</i>	07X10 ³	4X10 ²	01X10 ²
<i>Candida sp.</i> (Cellulase-producing)	09X10 ⁴	5X10 ³	

The data has been represented below graphically.

3.5. Effect of Pesticide exposure

The LC50 values at 96 hours of exposure were found to be 0.016 and 0.052 for Pendimethalin and Pretilachlor respectively.

No mortality was observed among any pesticide treated group of earthworms. But the number of gut microflora varied in different test conditions as summarized below in Table 7.

Table 7
Summarized effects of both the doses of pesticides on the isolates
from *Perionyx excavatus*

Isolates	Abundance/Presence/Absence				
	Control	PI1	PI2	PII1	PII2
<i>Bacillus cereus</i>	++++	+++	+	++++	++
<i>Enterococcus faecalis</i>	+++	+++	-	++	-
<i>Micrococcus luteus</i> (Cellulase-producing)	++++	+++	++	++	++
<i>Bacillus megaterium</i> (Cellulase-producing)	++++	+++	++	++	+
<i>Enterobacter cloacae</i> (Cellulase-producing)	++++	++	+	++	+++
<i>Aspergillus fumigatus</i>	++++	++	-	+	-
<i>Pseudallescheria boydii</i> (Cellulase-producing)	+++	-	-	-	-
<i>Streptomyces sp.</i> (Cellulase-producing)	+++	++	+	+	-
<i>Nocardia sp.</i>	+++	+	-	-	-
<i>Candida sp.</i> (Cellulase-producing)	+++	+	-	+	-
+ Presence; ++ Average growth; +++/more Abundance; - Absence					

For further confirmation of the effect of pesticide doses on the isolated microflora, CFU was calculated for each of the isolate and compared with the control (Table 8).

Table 8: CFU calculation of each isolate for pesticide exposure

Isolates	CFU/ml				
	Control	Pesticide I		Pesticide II	
		Dose 1	Dose 2	Dose 1	Dose 2
<i>Bacillus cereus</i>	243X 10 ⁵	127X 10 ⁵	86X10 ³	233X10 ⁵	54X10 ³
<i>Enterococcus faecalis</i>	423X10 ⁵	242 X10 ⁵	-	131X10 ³	-
<i>Micrococcus luteus</i> (Cellulase-producing)	156X10 ⁵	154X 10 ⁴	76X 10 ²	81X10 ²	63X10 ²
<i>Bacillus megaterium</i> (Cellulase-producing)	273X10 ⁴	214X10 ⁴	177X 10 ³	110X 10 ³	54X 10 ²
<i>Enterobacter cloacae</i> (Cellulase-producing)	194X10 ⁴	118X 10 ²	88X 10 ²	108X 10 ³	141 X 10 ³
<i>Aspergillus fumigatus</i>	23X10 ³	14X 10 ³	-	10X 10 ²	-
<i>Pseudallescheria boydii</i> (Cellulase-producing)	52X10 ²	-	-	-	-
<i>Streptomyces sp.</i> (Cellulase-producing)	16X10 ³	10X 10 ³	13X10 ²	7X 10 ²	-
<i>Nocardia sp.</i>	07X10 ³	5X10 ³	-	-	-
<i>Candida sp.</i> (Cellulase-producing)	09X10 ⁴	4X10 ²	-	5X10 ²	-

4. Discussion

Several novel cellulase-producing microbes from a wide variety of environments have been isolated and studied. The identification of novel cellulases from bacteria is currently a vast explored route to the improvement of biorefining industries. The CMC_{case} activity of the newly isolated cellulase from different microbes is much higher than the activity on Avicel or filter paper and this enzyme have maximum CMC_{case} activity at 60°C, pH 6.5. The high thermostability and slight acidic tolerance of this enzyme contributes to its potential for industrial use for the hydrolysis of soluble cellulose as well as activity on microcrystalline or other sources of cellulose (Wang et al., 2008). The rapid growth rate, enzyme complexity and extreme habitat variability of bacteria and fungi present an attractive potential for the exploitation of cellulases and hemicellulases present in the environment. For this, development of rapid and reliable methods for the isolation and screening of cellulases from microorganisms will provide a higher number of novel cellulases to be isolated for the industrial use. The enzymes isolated till date, are

not fully resistant to the harsh environmental conditions used in the bioconversion processes such as high temperature, metal, acidic or alkali pretreatments.

The ability to degrade cellulose is an important character of a wide variety of aerobic, facultative aerobic, anaerobic microbes. The impact of earthworms on metal and metalloid mobility within the soil can be explained by earthworm-induced changes in soil pH (Masscheleyn et al., 1991; Temminghoff et al., 1997; Martínez and Motto, 2000) or WEOC (Jordan et al., 1997; Temminghoff et al., 1997; Bauer and Blodau, 2009).

But there are less data when it comes to the impact of metal on the gut microflora of earthworm *Perionyx excavatus*. The microbes and earthworms play an important role in enhancing the nutrients in the soil by mineralisation through the secretion of various enzymes (Parthasarathi and Ranganathan, 1999; 2000; Vinotha et al., 2000), the humic acid increase in the vermicast sequesters elements like zinc from their complex forms, making them available for uptake by the plants (Parthasarathi and Ranganathan, 2002). The humic acid is known to be very reactive with metal ions due to the presence of their diverse functional groups (Aswathanarayana, 1999). Hence, this microbes-earthworms relationship throws light on the flux of nutrients, particularly trace elements.

The application of bacteria in producing cellulase is not being widely used despite of its higher growth rate compared to fungi. The cellulolytic property of some bacterial genera such as *Cellulomonas*, *Cellvibrio*, *Pseudomonas sp.* (Nakamura and Kppamura, 1982), *Bacillus*, and *Micrococcus* has also been reported. The production of these enzymes is closely controlled in the microorganisms and for improving its productivity these controls can be improved. Production of cellulase appear to depend upon a complex relationship involving a variety of factors like size of inoculum, pH value, temperature, presence of inducers, medium additives, aeration, growth time, and so forth (Immanuel et al., 2006).

In the present study, we observed the effect of the metal Zn^{2+} and two commonly used pesticides Pretilachlor and Pendimethalin on the cellulase-producing microbial flora of earthworm *Perionyx excavatus*. We exposed the earthworms in two different doses (Dose I and Dose II) of this metal and two doses of two pesticides; it was observed that this metal and pesticides have an overall detrimental effect on the gut microbes of the earthworm. But out of the 6 isolated cellulase-producing microbes, the higher dose of the metal was found to be dangerous to 4 of the isolates. These isolates are found to be producing a sufficient amount of cellulase when cultured in appropriate conditions. Further, we noted that the lower dose of the metal was enough to inhibit the growth of the bacterium *Bacillus megaterium*. Also, it has been observed that *Micrococcus luteus* and *Enterobacter cloacae* were not at all affected by the presence of metal in the media, rather their population increased when they were introduced to metal-polluted soil. The cellulase-producing fungal isolates were also affected when exposed to higher dose of the metal. No toxicity studies have been reported on *P. excavatus* in artificial soil. Despite heavy usage and environmental persistence, little information is available regarding the effects of Pendimethalin in non-target soil organisms (Belden *et al.*, 2005). The cellulase-producing fungus *Pseudallescheria boydii* was found to be absent even at the lower dose of the pesticides. *Candida sp.* showed susceptibility

towards the higher doses of the pesticides. Three cellulase-producing bacteria i.e. *Micrococcus luteus*, *Bacillus megaterium* and *Enterobacter cloacae* were found present in the presence of both the doses of the pesticides. From all the above results it may be concluded that the cellulase-producing bacteria *Enterobacter cloacae* and *Micrococcus luteus* were not highly affected by the presence of either metal or pesticides in the soil and hence they might play a great role in the effective production of enzyme cellulase. The results also showed that the metal and pesticides were proved to be more harmful for the gut fungal isolates than the bacterial isolates. The higher dose of pesticides were proved to be the most detrimental for the isolated microflora as most of the microbes were found to be absent when exposed to the higher dose of the pesticide and the metal.

Reduction in the population of these economical important microbes might pose a negative effect on the decomposition processes occurring within the soil. Also the use of commercial pesticides might prove to be the most detrimental for these microbes.

The study has been taken up as fungi are only being used for cellulase activity but the earthworm gut microflora contains a better mixture of bacteria and fungi for cellulase production. Hence, a study on such cellulase-producing microbes isolated from natural sources that are least affected by the presence of metal or pesticide contamination in the environment would be of greater help to the industries that require huge amount of cellulases.

Declarations

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These are not applicable in this work

- Animal Research (Ethics)
- Consent to Participate (Ethics)
- Plant Reproducibility
- Clinical Trials Registration
- Conflict of Interest

Consent to Publish (Ethics) We declare that this work has not been published elsewhere, either completely, in part, or in another form. The manuscript has not been submitted to another journal and will not be published elsewhere within one year after its publication in this journal.

Data Availability The given data will be available for all academic purposes.

Author Contribution Both the authors have their contributions in this present work.

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Figures



Figure 1

Plate showing cellulase-producing microbes on CMC agar plate

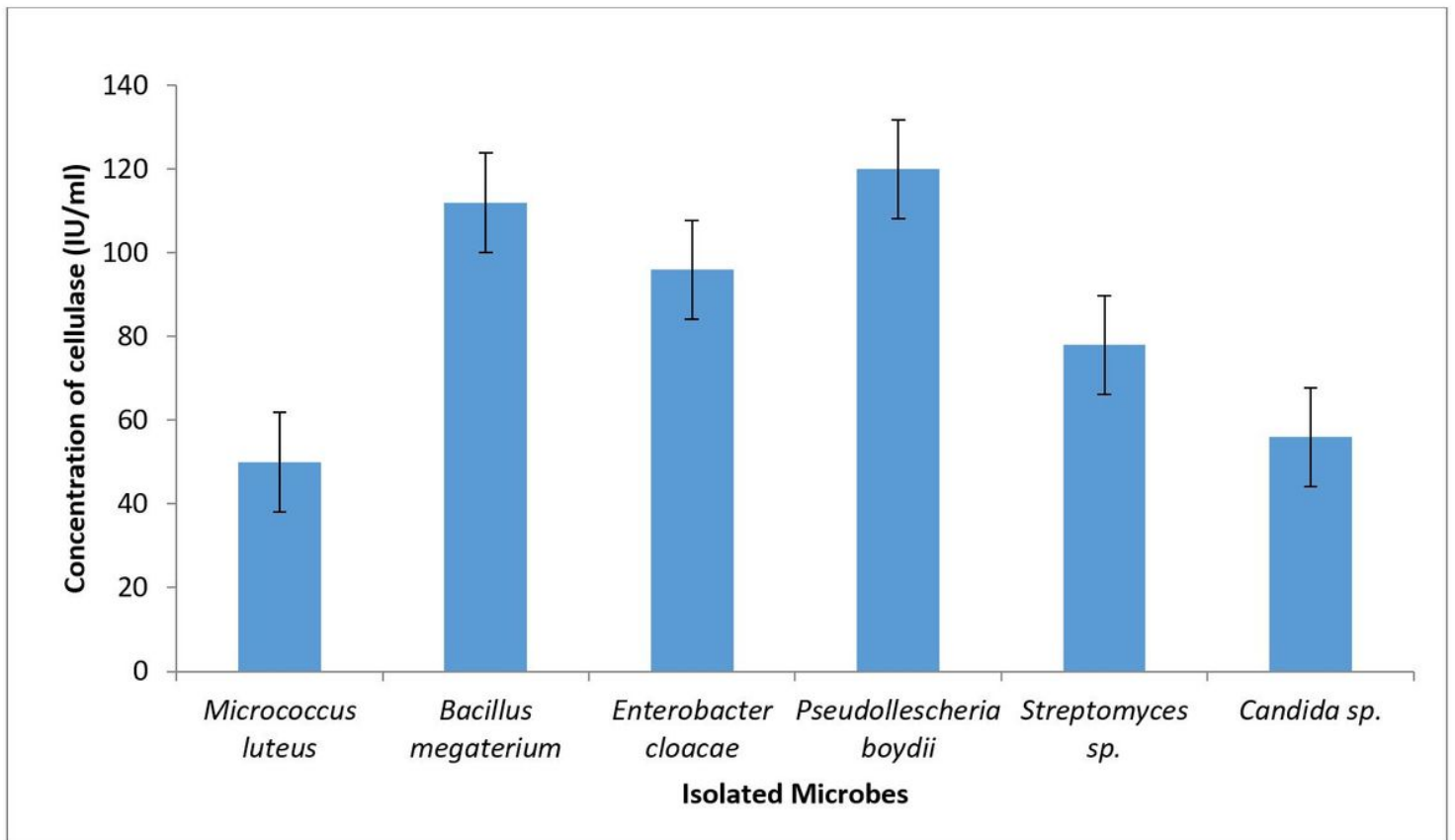


Figure 2

Graphical representation of the amount of cellulase produced by the isolated microfloral species

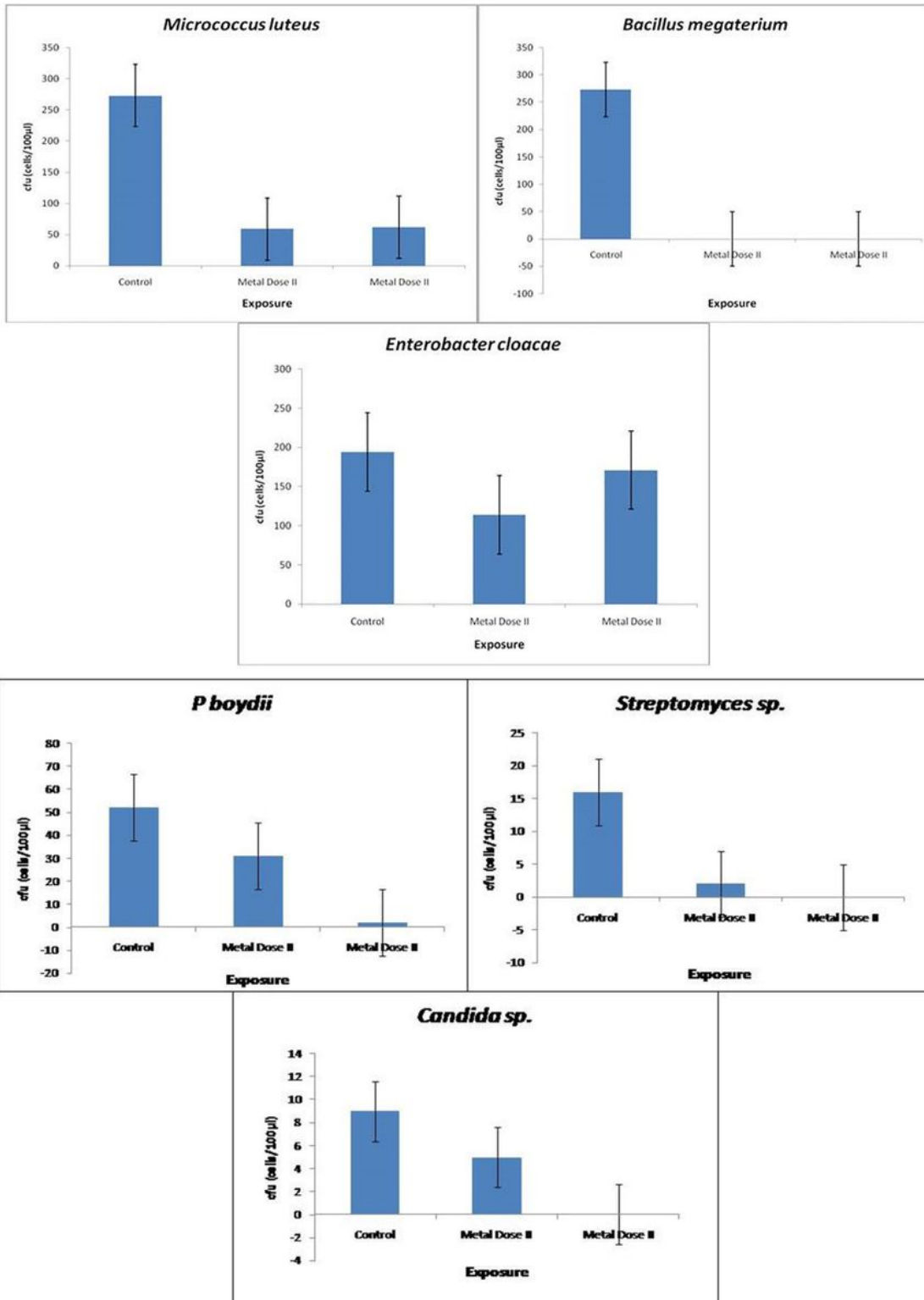


Figure 3

Bar diagrams showing the effect of metal Zinc on the CFU of the cellulase-producing microbes