Original Research Article

Heavy metal resistance properties of bacteria from different soil types in Horo Guduru Wollega, Ethiopia

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ABSTRACT

Background: The quality of life on earth is linked inseparably to the overall quality of the environment. Soil pollution with heavy metals has become a critical environmental concern due to its potential adverse ecological effects. The study explored the heavy metals resistance properties of bacteria isolated from fertilizer applied agricultural and non-agricultural soils.
Methods: The soil samples were collected from both fertilizer applied agricultural soils and non-agricultural soils. After identification and characterization of the isolates from both soil types, six (6) similar bacterial isolates were selected to screen for resistance against Cobalt (Co^+), Lead (Pb^{2+}), Cromium (Cr^{+3}), Mercury (Hg^{2+}), Nickel (Ni^{2+}), Cadmium (Cd^{2+}) and Zinc (Zn^{2+}) heavy metals. The minimum inhibitory concentration (MIC) for the bacterial isolates were determined by gradually increasing the concentration of heavy metals on agar plates until the isolates failed to show growth.
Results: The isolates from fertilizer applied agricultural soil showed the highest resistance against the selected heavy metals than those isolated from fertilizers not applied (nonagricultural) soils.
Conclusions: From this result it can be seen that fertilizer has significant role in influencing the heavy metal resistance properties of bacteria and these heavy metal resistant bacteria can be useful for the bioremediation of heavy metal contaminated environment.

Keywords: Fertilizer, Bacterial isolates, Soil

INTRODUCTION

Soil pollution with heavy metals has become a critical environmental concern due to its potential adverse ecological effects. According to study reports, heavy metals in soil may be found in one or more of the four forms: dissolved (in soil solution), exchangeable (in organic and inorganic components), as structural components of the lattices of soil minerals, as insoluble precipitates with other soil components.[1] The source of heavy metals in soil can be the industries (non-ferrous industries, iron, steel and chemical industries), agriculture (irrigated with polluted water, mineral fertilizers, contaminated manure, sewage sludge and pesticides containing heavy metals), from waste incineration, burning of fossil fuels and road-traffic.[2]

Since soil is one of the most important environments for microbes and is easily exposed to many pollutants, evaluating the effects of pollutants on the microbial population are very valuable. Currently, microorganisms are being used as potential bioindicators for the assessment of chemical risk to the ecosystem and effects of heavy metals on the growth of plants and

microorganisms have been investigated by several workers.[3] The aim of this research is to evaluate the extent of the heavy metals in the selected Agricultural soils by primary screening of bacteria for metal resistance.

The loading of ecosystems with heavy metals can be due to excessive fertilizer and pesticide use, irrigation, atmospheric deposition, and pollution by waste materials. The availability of accurate data on heavy metal contamination of agricultural soils and identifying heavy metal resistant bacteria is an essential requirement for removal of heavy metals from contaminated sites as well as for agricultural soil management. The current study was designed to assess the extent of heavy metals resistance properties of bacterial isolates in fertilizer applied agricultural soils and non-agricultural soils.

METHODS

Study area

The study was conducted at Horro Guduru Wollega. Horro Guduru Wollega is located in the west part of Ethiopia, 314 km west of Addis Ababa, 64 km to the North West of the main road from Addis Ababa to Nekemte. The zone is located between 09º29´N and 37º26´E, at an altitude of approximately 2296 major arena soccer league (m.a.s.l.), with a uni-modal rainfall ranging between 1200-1800 mm.[4] The sample of the study was taken from the three randomly selected woreda (Abbay Coman, Abe Dongoro and Amuru).

Soil sample collection

The soil samples were collected from three areas at a depth of 1-5 cm for both fertilizer applied agricultural soils and non-agricultural soils from each of the selected area and immediately transported to the laboratory for further study. The soil samples were mixed and the large soil particles were removed before sample processing.

Isolation and identification of heavy metal resistant bacteria

For isolation of heavy metal resistant bacteria, a serial dilution of the soil samples was done. The concentration of each heavy metal was maintained in the stock solution. The soil samples were spread plated on Nutrient Agar media supplied with heavy metals and incubated at 30°C for 3 days. After incubation, bacterial growth on the media was observed. Identification of the isolates was done on the basis of colony color and morphology, and standard biochemical tests like gram stain, Simmon's citrate utilization test, catalase test, and thyoglycolate test.[5] After identification of the isolates, similar isolates from both soil types (fertilizer applied agricultural soils and non-agricultural soils) were preserved for the determination of their minimum inhibitory concentration (MIC).

Determination of MIC of the heavy metals

MIC for all selected isolates were determined by growing the isolates on heavy metal incorporated media against respective heavy metal increasing the concentration of the heavy metals by 200 ppm (µg/ml) each time on agar plates until the isolates failed to show growth on the plate. For each of these heavy metals the culture growing on the last concentration was transferred to the next higher concentration by streaking on the plate until the isolate failed to show growth. The MIC was noted when the isolate failed to show growth on the plates.[6]

Data analysis

The data collected on the heavy metal resistance properties of the isolated species were analyzed using Microsoft Word Excel.

RESULTS

In this study soil samples were taken from fertilizer used soils and areas where fertilizer is not applied soils for each selected woreda. Totally, seven bacterial isolates from fertilizer applied agricultural soil and twelve isolates from fertilizer not applied (non-agricultural) soils were isolated. From both soil types six similar isolates were selected after identification and characterization of the isolates to screen for heavy metals resistance. Finally, these bacterial isolates were tested for their resistance properties against Co^+, Pb^{2+}, Cr^{+3}, Hg^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+} heavy metals.

Table 1: MIC for bacterial isolate from fertilizer applied agricultural soils.

Heavy Metals	*Pseudomonas* WF	*Proteus bulgaris* WF	*Bacillus* WF	*Acinetobacter* WF	*Arthrobacter* WF	*Klebsiella* sp. WF
Co	1200	1200	1200	1200	1000	1200
Pb	1000	800	1200	1200	800	1200
Cr	1200	1200	1200	1000	1000	1000
Hg	200	200	300	400	200	400
Ni	800	800	1400	1000	800	1200
Cd	300	300	300	400	200	500
Zn	1200	1200	1200	1200	1000	1200

WF: with fertilizer.

Table 2: MIC for bacterial isolate from fertilizer not applied non-agricultural soils.

Heavy metals	*Pseudomonas* WoF	*Proteus bulgaris* WoF	*Bacillus* WoF	*Acinetobacter* WoF	*Arthrobacter* WoF	*Klebsiella* sp. WoF
Co	400	400	400	400	400	400
Pb	400	400	400	400	400	400
Cr	800	400	400	400	400	500
Hg	200	200	200	200	200	300
Ni	400	400	400	400	200	600
Cd	200	300	200	200	200	200
Zn	400	400	400	400	400	400

WoF: without fertilizer.

Table 3: Number and percentage of bacterial isolates resistant to heavy metal at a concentration of 500 μg/ml and above (n=6).

Heavy metals	No. of bacteria	
	Fertilizer applied soil, N (%)	Fertilizer not applied soil, N (%)
Co	6 (100)	0 (0.0)
Pb	6 (100)	0 (0.0)
Cr	6 (100)	2 (33.33)
Hg	0 (0.0)	0 (0.0)
Ni	6 (100)	1 (16.67)
Cd	1 (16.67)	0 (0.0)
Zn	6 (100)	0 (0.00)

Table 4: Comparison of heavy metal resistance in the isolates from both soil types.

Analysis of variance						
Source of variation	SS	df	MS	F value	P value	F critical
Rows	4251190	6	708531.7	21.82	5.84E-14	2.24
Columns	5828571	11	529870.1	16.32	7.12E-15	1.94
Error	2143095	66	32471.14			
Total	12222857	83				

SS: sum of square; MS: total sum of square; df: degree of freedom.

The MIC of the isolates for each of the selected heavy metals were determined by gradually increasing the concentration of the heavy metal on nutrient agar plates in stepwise until the isolate failed to show growth. There was a decline in the growth of the isolates with increasing concentrations of the heavy metals in the media in contrast to the situation in the control i.e., 0.0 μg/ml of the metals where there was a profuse/heavy growth by all the isolates. The bacterial load decreased with the increase in concentration of heavy metals indicating toxic effect of the heavy metals on the growth of the isolates.

All (100%) of the isolates taken from fertilizer applied soil showed growth when the concentration of Co, Pb, Cr^{+3}, Ni and Zn were above 500 μg/ml and no isolates could tolerate Cd^+ and Hg^+ above 500 μg/ml (Table 1 and 2). In contrast to isolates from the fertilizer applied soil type, except for Cr 2 (33.33) and Ni, 1 (16.67) no any of the isolates from fertilizer not applied soils showed tolerance for the other four (Co, Pb, Hg, Cd and Zn) heavy metals.

The results from this study indicated that, the highest percentage of resistance was in agricultural soils used with fertilizers (Table 3). All bacterial isolates from fertilizer applied soils showed tolerance at least up to 800 μg/ml concentrations to five of the selected heavy metals except for Cd and Hg which are very toxic to all the selected isolates. In contrast to this, the isolates selected from the soils that have no fertilizer did not show resistant to the heavy metals at this concentration.

Heavy metal resistance variation in bacterial isolates from fertilizer applied soils and fertilizer not applied (nonagricultural) soils was determined above in Table 4. Statistical analysis showed that there is significant difference of heavy metal resistance among the isolates with confidence interval of 95% (p value <0.05).

Tables 1-4 show the comparison between similar isolates from fertilizer applied soils and fertilizers not applied soils. As shown in these tables all the isolates from fertilizer applied soils showed more resistance to every of

the selected heavy metals than those from fertilizer not applied soils.

DISCUSSION

Previous study reported that, however there were no differences in the pH, texture and organic matter content between soils contaminated with industrial effluent and uncontaminated soil samples, significant differences were observed for heavy metal content, namely, for Pb, Zn and Hg.[5] In the same manner, the resistance properties of the isolates from fertilizer applied soils are higher than the isolates from fertilizer not applied soils in the current study. Similar to this result, in other study by Oliveira et al, is stated that the soil heavy metal concentration increases after fertilization, which can affect the resistance properties of microbial communities in the soil.[7]

Atafar et al study showed that, fertilizer is important tools for global food security, but has undesirable effects on environment, human health and microbes. It was stated that among varied adverse effects of chemical fertilizers they held responsible for strongly influencing the properties of soil microbe.[8] Prashar et al in his study stated that there was a great difference between the agricultural soils and non-agricultural soils for the resistance not only for Pb, but also all selected heavy metals of this study.[9]

As indicated in the result, the highest percentage of resistance was in agricultural soils used with fertilizers, which agrees with different previous report of Kasra et al.[10] The high level of MIC in the first group (isolates from fertilizer applied soils) of this study was possibly due to the existence of heavy metals in the soils from different source including fertilizers which increase the population of heavy metal resistant bacteria. Bacteria exposed to high levels of heavy metals in their environment have adapted to this stress by developing various resistance mechanisms. Therefore, the finding of this study implies that the fertilizer has significant impact on the development of heavy metal resistance in the isolates. Furthermore, this implies that fertilizer increase the level of heavy metals in agricultural soils.

CONCLUSION

The identification of resistance against different metals may provide a useful tool for the estimation of heavy metal levels in the soil and for the simultaneous monitoring of several toxic pollutants in the environment. This study showed that all the bacterial strains isolated from agricultural soils with fertilizers applied had considerable resistance to different heavy metals than those from fertilizer not applied soils. This may illustrate that fertilizers can increase the level of heavy metals in agricultural soils, hence it needs to put standards on the heavy metal levels of organic fertilizers. Considering the

wide range of multiple-metal resistant at high concentrations by bacterial isolates of present study, these isolates could help in the effective bioremediation of heavy metal contaminated sites. On the basis of this result it can be recommended that evaluating the levels of heavy metals in the environment frequently is necessary and heavy metal content of organic fertilizers should be minimized as possible. Furthermore, the heavy metal resistant microbes can be used as indicator of environmental contamination with the heavy metals.

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