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Bioremediation Potential of Garbage of Enzymes From Fermented Organic Wastes on Selected Heavy Metal (Fe, Co, Zn, Cu) Concentrations on Crude Oil Polluted Soil

E. O. Wellington^{1,*} and I. E. ThankGod²

¹ Department of Chemical Science, Faculty of Basic and Applied Sciences, University of Africa, Toru-Orua, Nigeria

² Department of Biochemistry, Faculty of Science, University of Benin, Benin City, Nigeria

*E-mail address: ewellington41@yahoo.com

ABSTRACT

This exploration evaluated the bioremediation potential of garbage of enzymes (GE) from fermented organic wastes on Fe, Co, Zn, Cu concentrations on crude oil polluted soil. The natural gas polluted soil was self-possessed from Agbura community of Bayelsa State. The crude oil polluted were divided into 6 groups. Group 1 and 2 served as control and untreated soil samples respectively. Group 3 was treated with 30% of GE from fermented watermelon and pineapple wastes. Group 4 was treated with 50% of GE from plantain and banana wastes. Group 5 was treated with 70% of GE from plantain, watermelon, pineapple and banana wastes. Group 6 was treated with 100% of GE from plantain, watermelon, pineapple, and banana peels. Group 3, 4, 5, and 6 were treated for 30, 60, 90, 120, 150, and 180 days using 90 days GE from organic peel wastes. The Cu concentration in group 6 (91.05±0.0%) after treatment with 100% of GE were significantly higher than those of group 2 (17.34±0.02%). The Zn concentration in group 6 (83.25±0.03%) after treatment with 100% of GE were higher than those of group 2 (12.05±0.0%). The Co concentration in group 6(81.06±0.04%) after treatment with 100% of GE were higher than those of group 2 (17.33±0.02%). GE from organic peels demonstrated percentage Fe, Cu, Zn, and Co percentage removal from crude oil polluted soil after treatment, hence could be used as bioremediation tool in crude oil spill cleanup.

Keywords: Crude oil, heavy metals, garbage enzymes, pineapple, plantain, watermelon, banana peels

1. INTRODUCTION

Garbage enzyme (GE), product of fermented organic deteriorate comestibles, fruits peel, brown sugar and aqueous solution serve as a universal solvents for house and agricultural application. Garbage enzymes cannot be produced from any chemical substance. After ferment, a combination and stable ecological complex that possesses numerous roles emerges, producing three functionalities including: mortification, coalescence and biotransformation.

The experimentation of the amino acid entities of garbage enzyme presented that fundamental principal constituents are embodied in raw materials of plant and microbes, natural bioactive ingredients in plants, physiological materials and plenty micro-minerals are generated by fermentation, and less harmful chemicals.

Currently, garbage enzyme are progressively capitalized and adopted in aqueous and air restoration, soil restoration crop growth and quality augmentation, decomposition of chemical components such as chemical fertilizer and pesticide residues.

Exudes and fortuitous knock over occur periodically during the examination, manufacturing, clarification, transport and storage of petroleum and petroleum products in the Niger Delta region. The release of heavy metal pollutants in the environs, accidentally or anthropogenic activities is a main cause of environmental contamination. Soil inorganic or organic contaminants results in a high degree of environmental degradation which may elicit toxic consequences and chromosomal alterations.

The presence of inorganic pollutants in soil, air, and water has become a serious concern considering their harmful effects in very small quantities. Meanwhile, these pollutants are nonimperishable, bioaccumulate in tissues and are biomagnified through trophic level explained that disintegrating of geological understructure and volcanic eruptions can exude inorganic toxic minerals into the environment. Successive releases of inorganic pollutants into the food chain relies on their degree of levels and absorption by local flora and fauna while atmospheric deposition which has been shown to be also responsible for major reason of the presence of deposits in urban and sub-urban areas.

Inorganic elemental particles naturally exist in the soil, but industrial activities and unintentional spillage leads to high levels of these elements, considered deleterious to both plants and animals. The group of heavy metals such as; iron, manganes, cobalt, copper, zinc, and molybdenum, when present in levels slightly higher than normal values could produce negative consequences Terretial and aquatic organism including humans. The maximum degrees of thes inorganic minerals in soil, foodstuffs of plant origin should be kept at the strictest possible concentrations that is reasonably attainable by good practices of industries and taking into account the risks associated with food consumption.

Remediation of inorganic minerals in soil and water through traditional methods can be divided into chemical, physical and biological methods (Dhaliwal *et al.*, 2019; Tchounwou *et al.*, 2012). Chemical additives will result in secondary contamination, and the adoption of physical amendment technique is always restricted for high cost and complex equipment. Bioremediation method, which is environmental friendly and efficient, is indicating a large utility prospect in the field of inorganic contamination remediation.

This eventually emerge as principal technique of in-situ removal of inorganic soiled soil in recent times. However, there very few literature regarding the adoption of enzymes to decontaminate polluted soil. In addition, the soils in Agbura Community has been recently heavy metal concentration, posing environmental challenge. Adopting a method that is defined by microbial agents microbial could be efficacious in reducing the problems and conform to the concept of sustainable development.

In this study, garbage enzymes solution were produced from watermelon, pineapple, ripen banana, and plantain peel wastes and were used to amend soil quality. Through the pot experiment, the effects of different cock tail enzyme solution, their percentages on the crude oil contaminated soil and the absorption of inorganic minerals were evaluated. The cock tail enzyme solution is suitable for the amendment of soil and the best percentages were selected as the resource of peels of the organic wastes to provide technical guidance and scientific basis for the utilization of resources and the acceleration of soil fertility improvement and pollution remediation in the region.

2. MATERIALS AND METHODS

2. 1. Area of Study and Sampling

Agbura community was choiced for in the present investigation because the spillage took place in recent time. The community is located very close to the capital city of Bayelsa State. The population of the city is 1200. It one of the known villages in Bayelsa State, with 217.7 mm annual precipitation, 11.8 °C temperature and 46% humidity.

The features of the soil in the community was sandy loam consisting of 80 % sand, 12 % loam, 6 % sludge and 2 % organic substances and with pH 6.8. The polluted soil sample was obtained having a characteristic of black hardened coloration and sealed in sterile polytene vessles, and was transported to Biochemistry Laboratory at University of Africa Toru-Orua for evaluation. The sample was stored at adequate temperature before experimental work.

3. 2. Sample Preparation

Exactly, 200 g of the samples was measured into three different vessels, adopting analytical balance. Also, 500 ml of deionized water was measured and added into the vessels containing the sample and it was mixed vigorously.

2. 3. Garbage Enzyme (GE) Preparation

GE was produced from three organic substances such as ripened pineapple (PA), watermelon (WA), plantain (PL), banana (BN) peels and brown or molasses sugar in ratio 10: 3: 1 respectively. The organic substances were allowed to undergo ferment for the period of 90 Days. The three organic substances (pineapple peels, watermelon, plantain and banana peel wastes) were obtained from Swali market Yenagoa.

The water used was fetched from the University of Africa undergraduate hostel, 12 L corresponding to 12 kg of water was carefully measured into a clean vessel, 1.2 kg of brown sugar was dissolved into the water to form sugar solution while 3.6 kg of the each of the four organic peel wastes were weighed and poured into the sugar solution. The mixture were thoroughly stirred together for proper combination and allowed to under fermentation for 90 days.

The organic solution harvested after fermentation is called a crude enzyme solution which contain different extracellular enzymes. Extracellular enzymes are the enzymes that are secreted by microbial agents which enter the aqueous phase during an aerobic submerged fermentation

process (Elena and John, 2004). According to Chaîneau *et al.* (2004), cock tail enzymes are produced and harvested during aerobic fermentation of organic matter

2. 4. Sample Treatment

The samples were separated into 6 groups treated as group 1-6. The groups were prepared and kept under shade to dry. The sample in each group was then oven at 105 °C, while 3 g of the sample was weighed into a platinum crucible, was ashed at 450- 500 °C and cooled at 25 °C in desiccators. Each sample was mixed with 5.0 ml of 20 % HCl and the solution was transferred a 100 ml volumetric flask. Each mixture was rinsed using deionized water and delivered into the flask, made up to the mark with deionized water and stirred for thorough mixing.

The heavy metal levels were then analyzed adopting AAS based on the method of the Association of Official Analytical Chemist.

2. 5. Experimental Design

The samples were divided into groups. Group 3 to 6 were amended using cock tail enzyme solution as presented below.

GROUP 1: Non-polluted soil sample, serving as control

GROUP 2: Polluted and untreated soil sample, serving as untreated soil

GROUP 3: Polluted and treated with GE 730t/hectare (30%) from WA+PA for 30, 60, 90, 120, 150, and 180 days

GROUP 4: Polluted and treated with GE 730t/hectare (50%) from PL+BN for 30, 60, 90, 120, 150, and 180 days

GROUP 5: Polluted and treated with GE140t/hectare (70%) PL+WA+PA+BN for 30, 60, 90, 120, 150, and 180 days

GROUP 6: Polluted and treated with GE1460t/hectare (100%) PL+WA+PA+BN for 30, 60, 90, 120, 150, and 180 days

At the end of the duration for each group, the soil sample carried to the laboratory for analysis based on standard methods.

2. 6. Determination of Heavy Metal Concentrations

Exactly, 1.0 g of each sample was measured into 50 ml beakers and was mixed with acids (HNO₃ and HClO₄) in 5:1 ratio. The sample is kept throughout the night for total digestion. The digestion was displayed at 190 °C for 1.5 h, which was then cooled and transferred into 100 ml volumetric flask. The mixture was then made up to a final volume up to the mark with deionized water. The Heavy metal levels were evaluated using AAS atomic in three data points.

2. 7. Statistical analysis

The investigation was structured, totally randomized using 6 treatments and 3 data points. Effects of the treatments were studied by analysis of variance with the help of statistical package and mean separation was tested by Tukey HSD.

3. RESULTS

Table 1-4 indicate the percentage removal of selected heavy metals on crude oil polluted soil samples treated with garbage enzyme (GE) solution.

Group	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	1.14±0.02 ^a	6.13 ± 0.03^{k}	$8.37{\pm}0.04^{k}$	$9.52{\pm}0.02^{k}$	11.16±0.02 ^p	13.03±0.03 ^p
Group 2	1.85±0.03 ^b	2.72±0.02 ^b	4.25 ± 0.03^{k}	$6.93 {\pm} 0.02^{p}$	13.06±0.03 ^p	17.34±0.02 ^p
GE WA+PA (30%)	7.03 ±0.03 ^c	16.25±0.03 ^f	21.82±0.02 ^f	25.14±0.03 ^f	27.13±0.03 ^f	35.85±0.04 ^f
GE PL+BN (50%)	5.74 ±0.03 ^c	$8.25 \pm 0.03^{\text{ f}}$	12.05±0.04 ^f	14.24±0.03 ^f	18.05±0.04 ^f	23.14±0.03 ^f
GE PL+WA+PA+BN (70%)	12.66±0.03°	21.05±0.04 ^f	28.25 ± 0.04^{f}	37.85±0.04 ^f	49.34±0.03 ^f	71.04±0.03 ^f
GE PL+WA+PA+BN (100%)	10.46±0.03°	14.05±0.05 ^f	23.64±0.04 ^f	34.43±0.03 ^f	51.15±0.03 ^f	61.05±0.04 ^f

Table 1. Percentage removal of Iron (Fe) on crude oil polluted soil samples treated with garbage enzyme (GE) (n=3)

GE = garbage enzymes, WA = watermelon, PA = pineapple, PL = plantain, BN = banana. Values are reported in SEM. Values with superscript b are statistically different from group 1 at (P < 0.05) down the groups. Values with superscript c are statistically different from group 1 and 2 at (P < 0.05) down the groups. Values with superscript k are statistically similar at (P < 0.05) across the treatments. Values with superscript f are statistically different from group 1 and 2 at (P < 0.05) across the periods of treatments.



Groups

Fig. 1. Percentage removal of Iron (Fe) in polluted soil samples treated with garbage enzyme (GE).

Group	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	4.25±0.03 ^a	8.66±0.03	14.33±0.03 ^p	$23.84{\pm}0.03^{p}$	31.04±0.03 ^p	39.25 ± 0.04^{p}
Group 2	$4.94 \pm 0.04^{\text{b}}$	4.04±0.02 ^b	6.37 ± 0.03^{p}	9.25 ± 0.03^{p}	17.65±0.04 ^p	21.61±0.01 ^p
GE WA+PA (30%)	19.84±0.04 ^c	27.75±0.04 ^f	34.75±0.04 ^f	48.24±0.02 ^f	64.06±0.03 ^f	73.79±0.03 ^f
GE PL+BN (50%)	12.95±0.03 ^c	$24.23{\pm}0.04^{\rm f}$	35.75±0.06 ^f	44.06±0.05 ^f	51.84±0.03 ^f	66.24±0.05 ^f
GE PL+WA+PA+BN (70%)	14.55±0.04 ^c	27.05 \pm 0.04 ^f	34.75 ± 0.04^{f}	39.55±0.04 ^f	43.84±0.03 ^f	67.06±0.03 ^f
GE PL+WA+PA+BN (100%)	2.96 ± 0.04^{c}	8.54 ± 0.02^{f}	45.04±0.03 ^f	56.84±0.04 ^f	$70.43{\pm}0.03^{f}$	$91.05 \pm 0.0^{\rm f}$

 Table 2. Percentage removal of copper (Cu) on crude oil polluted soil samples treated with garbage enzyme (GE) (n=3)

GE = garbage enzymes, WA = watermelon, PA = pineapple, PL = plantain, BN = banana. Values are reported in SEM. Values with superscript b are statistically different from group 1 at (P < 0.05) down the groups. Values with superscript c are statistically different from group 1 and 2 at (P < 0.05) down the groups. Values with superscript k are statistically similar at (P < 0.05) across the periods of amendments. Values with superscript f are statistically different from group 1 from group 1 and 2 at (P < 0.05) across the periods of amendments. Values with superscript f are statistically different from group 1 and 2 at (P < 0.05) across the periods of amendments.



World News of Natural Sciences 55 (2024) 102-118

Fig. 2. Percentage removal of copper (Cu) in polluted soil samples treated with garbage enzyme (GE).

World News of Natural Sciences 55 (2024) 102-118

Group	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	1.36±0.03ª	$2.45{\pm}0.03^{p}$	3.45 ± 0.04^{k}	16.94±0.04 ^p	22.73±0.03 ^p	28.62±0.03
Group 2	3.26±0.04 ^b	3.64±0.03 ^b	4.37±0.03 ^p	6.36±0.04 ^p	28.24±0.03 ^p	12.05±0.03 ^p
GE WA+PA (30%)	12.33±0.03°	15.01±0.02 ^f	22.75±0.04 ^f	31.94±0.04 ^f	41.22±0.03 ^f	53.65±0.04 ^f
GE PL+BN (50%)	$6.25 \pm 0.03^{\circ}$	8.93±0.04 ^f	17.55±0.04 ^f	27.85±0.03 ^f	44.05 ± 0.04^{f}	53.44±0.03 ^f
GE PL+WA+PA+BN (70%)	8.85± 0.04 °	16.25±0.03 ^f	32.02±0.05 ^f	46.33±0.04 ^f	59.06±0.05 ^f	73.14±0.03 ^f
GE PL+WA+PA+BN (100%)	11.57±0.02°	$22.3\pm0.02^{\text{ f}}$	37.76±0.04 ^f	58.02±0.03 ^f	65.46±0.04 ^f	83.25±0.03 ^f

Table 3. Percentage removal of zinc (Zn) on crude oil polluted soil samples treated with garbage enzyme (GE) (n=3)

GE = garbage enzymes, WA = watermelon, PA = pineapple, PL = plantain, BN = banana. Values are reported in SEM. Values with superscript b are statistically different from group 1 at (P < 0.05) down the groups. Values with superscript c are statistically different from group 1 and 2 at (P < 0.05) down the groups. Values with superscript k are statistically similar at (P < 0.05) across the periods of the amendments. Values with superscript f are statistically different from group 1 from group 1 and at (P < 0.05) across the periods of the amendments. Values with superscript f are statistically different from group 1 and at (P < 0.05) across the periods of the amendments.



World News of Natural Sciences 55 (2024) 102-118

Fig. 3. Percentage removal of zinc (Zn) in polluted soil samples treated with garbage enzyme (GE).

Group	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	6.33±0.01 ^a	17.83±0.05 p	27.93±0.0 ^p	44.85 ± 0.04^{p}	61.04±0.04 p	77.64±0.03
Group 2	0.75 ± 0.04^{b}	1.85±0.04 ^b	3.25±0.0 ^p	7.52±0.03 ^p	11.04±0.02	17.33±0.02
GE WA+PA (30%)	36.93±0.02 °	44.81±0.02	48.24±0.0 ^f	55.02±0.05 ^f	64.25±0.03 f	79.04±0.04
GE PL+BN (50%)	13.93±0.03 °	18.31±0.02	26.53 ± 0.0^{f}	38.94±0.04 ^f	51.65±0.05 f	68.34±0.02
GE PL+WA+PA+BN (70%)	5.72±0.03 °	15.23±0.04	27.34 ± 0.03	47.93±0.4 ^f	54.22±0.01	65.84 ± 0.03
GE PL+WA+PA+BN (100%)	8.35±0.03°	30.07±0.04 f	40.55±0.04 f	54.82±0.03 ^f	71.24 ± 0.05	81.06±0.04 f

Table 4. Percentage removal of cobalt (Co) on crude oil polluted soil samples treated with
garbage enzyme (GE) (n=3)

GE = garbage enzymes, WA = watermelon, PA = pineapple, PL = plantain, BN = banana. Values are reported in SEM. Values with superscript b are statistically different from group 1 at (P < 0.05) down the groups. Values with superscript c are statistically different from group 1 and 2 at (P < 0.05) down the groups. Values with superscript f are statistically different from group 1 and 2 at (P < 0.05) across the periods of the amendments

World News of Natural Sciences 55 (2024) 102-118



Groups

Day 120

77.64

■ Day 180

Day 90

Day 30

Day 60

4. DISCUSSION

Table 1 indicated the percentage decontamination of iron (Fe) in polluted soil samples treated with garbage enzyme (GE) from fermented organic peel wastes. The crude oil polluted soil treated with 30% of fermented organic waste from watermelon + ripened pineapple peels for 30 days were considerably higher than those of group 1 and 2 (Table). The percentage break down of iron on the crude oil contaminated soil by fermented garbage enzymes at 50 % ripened plantain + banana peels for 30 days were considerably higher than those of group 1 and 2 and similar result also occurred with those treated with 70 and 100% of plantain +watermelon+ banana peels for 30 days (Table 1).

The percentage degradation was observed to higher on soil sample treated with 70% of garbage enzymes fermented from plantain + banana peels followed by 100% treatment with plantain + pineapple+ pineapple peels while the least was 30% treatment using garbage enzymes from watermelon + pineapple peels (Table 1). The percentage decontamination of iron on crude oil polluted soil treated with 30% of fermented garbage enzymes from watermelon + pineapple peels, 70% of ripened plantain + watermelon + banana peels and 100% plantain + watermelon + banana peels for 60, 90, 120, 150, and 180 days was significantly higher than those of group 1 and 2 (Table 1). The percentage removal of iron levels in the sample increases as the days increases (Table 1). The significant percentage removal of iron levels on crude oil polluted soil by watermelon, ripened plantain, banana, and pineapple peels could be due increased garbage enzymes (protease, lipase, catalase, and amylase activities) degradation secreted by microbial agents in present in the harvested organic solutions. This results agrees with the report of Indo *et al.* (20121) on the biocatalytic remediation of used motor oil-contaminated soil by fruit garbage enzymes.

Table 2 shows the percentage removal of copper (Cu) in polluted soil samples treated with garbage enzyme (GE) from fermented organic peel wastes. The levels of copper in soil sample treated with 30%, 70% and 100% of garbage enzymes from fermented watermelon + pineapple, ripened plantain + watermelon + banana, and ripened plantain + watermelon + banana peel wastes respectively, for 90, 120, 150, and 180 days were higher than group 1 and 2 (Table 2). The garbage enzyme catalytic percentage abolition of copper ion in the sample after treatment using 30, 50, and 70% of watermelon + pineapple, ripened plantain + watermelon + banana, for 30 and 60 days respectively, were higher than those in sol soil sample treated with 100% of ripened plantain + watermelon+ banana peel wastes. The percentage removal of copper ion by garbage enzymes from fermented peel wastes is suggestive that soil abnormally high copper concentrations can be cheaply reduced by garbage enzymes from fermented organic peels wastes. Jing *et al.* (2021) in their investigation on simultaneous abolition of Cu and egradation of BDE-209 with soil microbial fuel cells, reported similar removal of copper ion from crude oil polluted soil.

Table 3 shows the percentage removal of zinc (Zn) in polluted soil samples treated with garbage enzyme (GE) from fermented organic peel wastes. The levels of zinc (Zn) in soil sample treated with 30%, 70% and 100% of garbage enzymes from fermented watermelon + pineapple, ripened plantain + watermelon + banana, and ripened plantain + watermelon + banana peel wastes respectively, for 90, 120, 150, and 180 days were remarkably higher than those of group 1 and 2 (Table 3). The levels of zinc (Zn) soil sample treated with 30% enzymes from watermelon and pineapple peels was observed to be outstandingly higher than those treated with 50% of garbage enzymes from ripened plantain + banana peel wastes for 30, 60,

90, 120, 150, and 180 days (Table3). The levels of zinc (Zn) in soil sample treated with 70% of garbage enzymes from ripened plantain +watermelon + banana peel wastes was observed to be remarkably higher than those treated with 50% of garbage enzymes from ripened plant and banana peels for 30, 60, 90, 120, 150, and 180 days (Table 3). The garbage enzyme catalytic percentage removal of zinc (Zn) ion in the sample after amendments using 70 and 100% watermelon + pineapple, ripened plantain+ banana, an ripened plantain + watermelon + banana, for 30 to 180 days respectively, were higher than those in sol soil sample treated with 30 and 50% of ripened plantain + watermelon+ banana peel wastes. The significant percentage removal observed on zinc ion after treatment with garbage enzymes formulated from ripened plantain, banana, watermelon and pineapple peel wastes in suggestive these organic waste could potential sources of bioremediation of metal pollutants in crude spill. Essien *et al.* (2015) on the impact of cow dung augmentation for remediation of crude oil polluted soil by *Eleusine indica*, reported similar results.

Table 4 shows the percentage removal of cobalt (Co) in polluted soil samples treated with garbage enzyme (GE) from fermented organic peel wastes. The levels of copper in soil sample treated with 30%, 70% and 100% of garbage enzymes from fermented watermelon + pineapple, ripened plantain + watermelon + banana, and ripened plantain + watermelon + banana peel wastes respectively, for 90, 120, 150, and 180 days were appreciably higher than those of group 1 and 2 (Table 4).

The levels of copper in soil sample treated with 30% enzymes from watermelon and pineapple peels was observed to be remarkably higher than those treated with 50 and 70% of garbage enzymes from ripened plantain + banana peel wastes and ripened plantain + banana + watermelon peel wastes respectively, for 30, 60, 90, 120, 150, and 180 days (Table 4). The levels of cobalt (Co) in soil sample treated with 50% of garbage enzymes from ripened plantain + banana peel wastes was observed to be outstandingly higher than those treated with 70% of garbage enzymes from ripened plantain + watermelon + banana peel wastes for 30, 60, 90, 120, 150, and 180 days (Table 4).

The garbage enzyme catalytic percentage removal of cobalt (Co) ion from the crude oil polluted soil, after treatment with 100% watermelon + pineapple, ripened plantain+ banana, an ripened plantain + watermelon + banana, for 150 to 180 days respectively, were higher than those in sol soil sample treated with 30, 50 and 70% of ripened plantain, watermelon, banana, watermelon peel wastes. This results agrees with the results of Owabor et al. (2011) on remediation of cobalt contaminated soil using manure, clay, charcoal, zeolite, calcium oxide, main crop (*Hordeum vulgare* L.), and after crop (*Synapis alba* L.).

5. CONCLUSION

Application of garbage enzymes from fermented fruit organic peel wastes have been demonstrated to immobilize and degrade metal agents in soil. This immobilization method of garbage enzymes might be used to lower levels of heavy metals in crude oil contaminated soil. In this study, fermented garbage enzyme solution from organic waste peels demonstrated varying percentage removal of Fe, Cu, Zn, and Co ion removal in crude oil polluted soil after 180 days treatment, hence could be used as bioremediation tools.

Note

This study demonstrated the effectiveness of garbage enzyme solution from fermented organic wastes in the bioremediation of selected heavy metals in polluted soil. This concept should be carefully studied in the light of current bioremediation techniques so that they can be accepted as appropriate bioremediation techniques.

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