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A STUDY ON THE IMPACT OF EXPIRED CLONAZEPAM TABLET ON SOIL QUALITY

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ABSTRACT

The use of medicines has been increased across the globe over the past century and continue to increase in the coming years due to the discovery of new treatments to cure recent diseases and various health disorders. Many pharmaceutical wastes are released into the environment by households, hospitals, and other activities. The unwanted drug disposal will be an issue in the landfill soil due to improper disintegration. Improperly handled pharmaceutical wastes will enter the landfills. Along with these wastes, some may subject for segregation and others may not. Based on the above concept, the current research has been done. To study the impact of expired tablets, a pilot study has been carried out to determine the impact of regularly discarded tablets on the landfill soils. For the study, expired Clonazepam tablet was used. This tablet was selected based on the survey carried on the types of pharmaceuticals disposed along with the households' wastes. Also, organoleptic characterization of expired clonazepam tablet was conducted. During the present study, the expired clonazepam tablet was added at concentrations of 1g, 5g, and 10g to the landfill soil sample and kept for observations for 30 days. The soil samples were collected and subjected for extraction to analyse various physico-

chemical parameters. From the study, it was observed that, the bulk density, particle density, water holding capacity and moisture content present in soil was higher than the normal range. The chemical parameters like phosphorus, calcium, magnesium, sulphate, available nitrogen and potassium values were found to be increase with the increase in the number of days and the clonazepam concentration.

Keywords: Clonazepam Tablet, Expired Drug, Physico-chemical properties, Soil Quality

I. INTRODUCTION:

Pharmaceuticals play a significant role in the overall well-being of living organisms. Due to its persistence in the environment, it has an adverse effect on the environment. The presence of various pharmaceutical compounds was observed by many researchers from their study in various environmental conditions. As a result, the present environmental research is focusing on how pharmaceutically active substances can contaminate natural resources. Because of the anthropogenic usage huge quantity of pharmaceuticals are entering into the sewage system **[1]**. One of the most significant wastes that contribute to pollution are municipal solid trash. They pollute the aquatic resources, soil health and agricultural production. Further it affects the soil microbial biomass **[2]**. Many pharmaceuticals will have negative effects due to its chemical composition. For instance, certain drugs may result in microbial resistance, which shows acute toxic effect or physiological functions of humans **[3]**.

Pharmaceutical chemicals in soil cause phytotoxic effects on plants. Due to the presence of toxic compounds in the soil, it inhibits the growth of plants. In certain treatments, the presence of pharmaceutical in soil matrices will change the pH of the soil over the time. The effects of unused or expired medicines on public health and environment are very much critical due to their toxicity, including genotoxicity and mutagenicity **[4]**. Among the various pharmaceuticals, benzodiazepines are a class of compounds that has the most psychoactive pharmaceuticals. The most commonly used benzodiazepines are clonazepam, diazepam, lorazepam, alprazolam and temazepam. The benzodiazepines share a common ring structure with individual benzodiazepines having variations in the ring substitution, which affects their respective physico-chemical properties and pharmacokinetic profile. The weak basic physico-chemical characteristics that benzodiazepines typically exhibit will ultimately determine how they behave in the environment **[5]**. With this concept, the present study is aimed at to know the effect of expired clonazepam tablet on soil quality.

II. MATERIALS AND METHODOLOGY:

In the present study, a pilot study has been done to identify the frequently used or disposed tablets that are having a negative impact on the landfill soil quality. During the survey, it was observed that, expired clonazepam tablet was found disposed along with domestic wastes. For the experiment, expired clonazepam tablets were collected and crushed to make fine powder. This powder is added to the soil samples at different concentrations to study its impact.

2.1 Collection of Soil Sample from the Landfill:

A composite soil samples were collected from the landfills of Mysore. The soil samples were air dried and sieved through 2mm mesh sieve and stored in polythene bags until analysis is done. The soil analysis was done as per the standard procedure.

2.2 Chemical composition of clonazepam (I.P 0.5mg) tablet and its usage: The chemical formula for clonazepam is 1,3-Dihydro-2H-1,4-benzodiazepin-2-one,

where positions 5 and 7 hydrogens are substituted by 2-chlorophenyl and nitro groups, respectively. The drug is used for the acute treatment of panic disorder, epilepsy, and nonconvulsive status epilepticus. The drug also has many off-label indications, including restless leg syndrome, acute mania, insomnia, and tardive dyskinesia.

2.3 Experimental Procedure: The processed soil samples were transferred to plastic pots, capacity of one kilogram. To this soil, the expired powdered clonazepam tablets was added at different concentration such as 1g, 5g, 10g with three trails each and kept for observation for 30 days. After the addition of the expired tablet, a tracer amount of water was added frequently to maintain the moisture level in the pots. At an interval of 10 days, the soil samples were taken out and the physico-chemical parameters were analyzed.

2.4 Organoleptic characterization of Expired Tablet:

Along with the physico-chemical characteristics, in order to compare the correlation between the influence of the expired clonazepam tablet on soil properties, the general organoleptic properties of the tablet were studied to ensure the quality and efficacy of the expired tablet. The organoleptic properties include, drug content, dosage of the drug, therapeutic class of the drug, hardness test, friability test, disintegration and dissolution test has been done and the results are presented in following tables.

III RESULTS AND DISCUSSIONS:

Table 1: Physical Characterization of Landfill Soil Sample Treated with Expired Clonazepam [I.P 0.5mg] with Different Concentrations						
Days	Soil Samples treatedwith different concentrations of clonazepam	Moisture Content(%)	Water HoldingCapacity (%)	Bulk Density(Mg/m^3)	Particle Density(Mg/m^3)	Porosity (%)
Soil Quality Standards		1-1.65	-	1-1.65	2-2.65	30-65
10^{th} Day	1g	4.92	39.31	1.18	1.923	38.01
	5g	5.27	40.56	1.18	2.044	38.19
	10g	5.28	41.26	1.2	2.136	41.01
20^{th} Day	1g	5.49	40.33	1.21	1.915	41.42
	5g	6.24	41.63	1.24	2.089	41.867
	10g	6.29	41.75	1.25	2.22	43.451
30^{th} Day	1g	6.43	45.88	1.278	2.33	45.146
	5g	6.81	47.97	1.286	2.45	47.49
	10g	7.13	48.41	1.291	2.49	48.143

Note: The experimental values are average values of 3 trials

Table 2: Characterization of Landfill Soil Sample Treated with Expired Clonazepam [I.P 0.5mg] with different concentrations (Average values of three trials of each concentration)																
Days	Soil Samples treated with Clonazepam	pH	EC (ds/m)	Ca^{2+} (ppm)	Mg^{2+} (ppm)	Cl^- (%)	OC(%)	N^+ (Kg/ha)	K^+ (Kg/ha)	P (Kg/ha)	SO_4^{2-} (ppm)	Zn^{2+} (ppm)	B^{2+} (ppm)	Fe^{2+} (ppm)	Mn^{2+} (ppm)	Cu^{2+} (ppm)
Soil Quality Standards		6.5-7.5	1-2	700-36000	1200-15000	0.01 – 0.99	0.5-0.75	240-480	110-280	10-25	8-30	0.6-1	0.5-1	4-9	2-4	0.2-0.3
10^{th}Day	1g	7.15	1.03	390	32	0.73	0.86	244.3	634	162	28.45	2.7	0.38	30.16	7.14	1.47
	5g	7.27	1.3	408	32	0.80	0.88	259.2	639	180	28.71	2.94	0.42	30.37	7.22	1.75
	10g	7.44	1.36	413	32	0.85	0.90	280.1	652	183	46.49	3.65	0.44	30.51	12.49	2.52
20^{th}Day	1g	7.46	1.43	1812	660	0.90	0.98	316.3	1763	541.7	110.01	3.83	0.48	32.71	14.17	2.95
	5g	7.49	1.45	1876	689	0.94	1.10	339.3	1795	640.3	127.63	3.87	0.53	33.24	17.5	3.21
	10g	7.61	1.46	2522	707	0.97	1.12	420.3	1879	1034	155.23	4.01	0.62	34.51	25.91	3.17
30^{th}Day	1g	7.61	1.52	2575	746	1.03	1.18	443.0	2280	1604	946.7	4.03	0.62	35.22	32.71	4.04
	5g	7.64	1.56	2834	760	1.10	1.32	466.3	2738	1807	1026.3	4.09	1.1	38.20	34.51	4.26
	10g	7.68	1.60	2975	772	1.17	1.52	522	3487	1853	1087.3	4.27	1.21	40.43	34.59	4.27

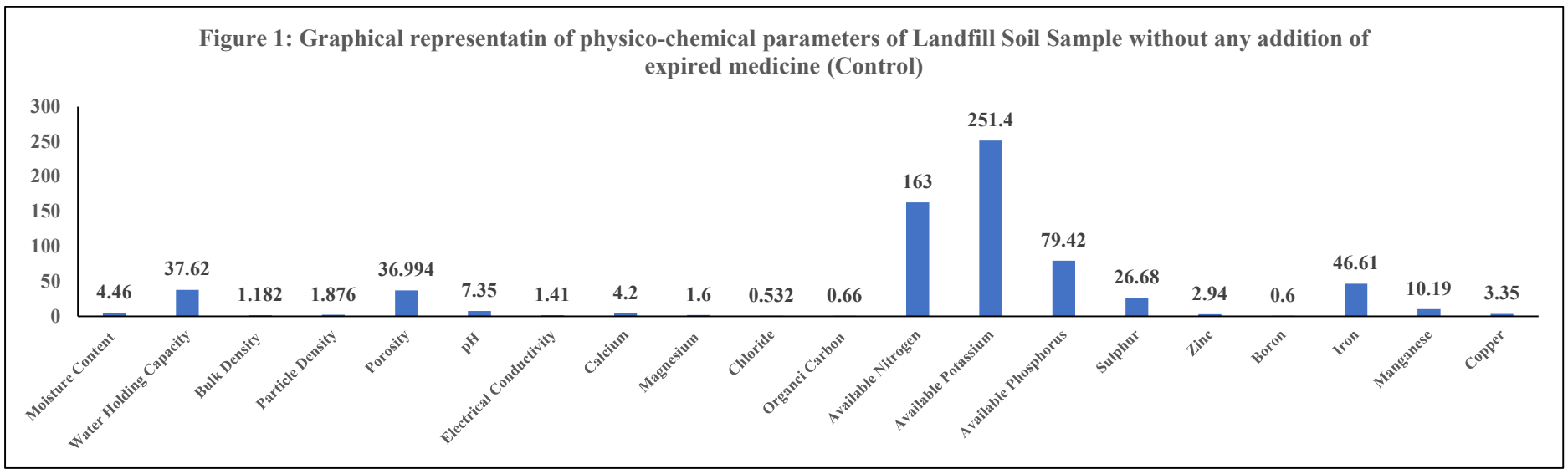


	Table 3: Physico-chemical Characterization of Landfill Site Soil Before the Addition of Expired Clonazepam Tablet (Control)																		
Parameters	MC	WHC	BD	PD	Porosity	pH	EC	Ca^{2+}	Mg^{2+}	Cl^-	OC	N^+	K+	P	SO_4^{2-}	Zn2+	B^{2+}	Fe^{2+}	Mn^{2+}	Cu^{2+}
	4.46	37.62	1.182	1.876	36.994	7.35	1.41	4.2	1.6	0.532	0.66	163	251.40	79.42	26.68	2.94	0.6	46.61	10.19	3.35

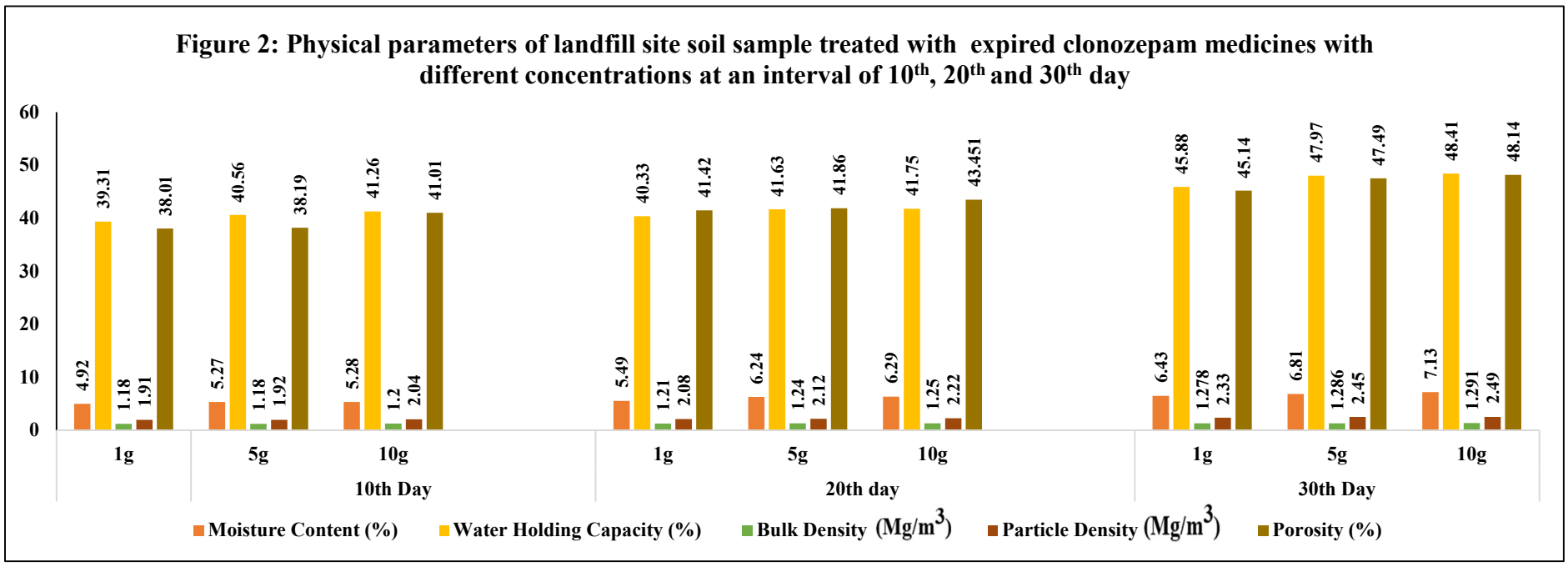


Figure 3: Graphical Representation of chemical parameters such as pH, EC, Cl^- and OC of landfill site soil sample treated with expired clonozepam medicines with different concentrations at an interval of 10^{th}, 20^{th} and 30^{th} day

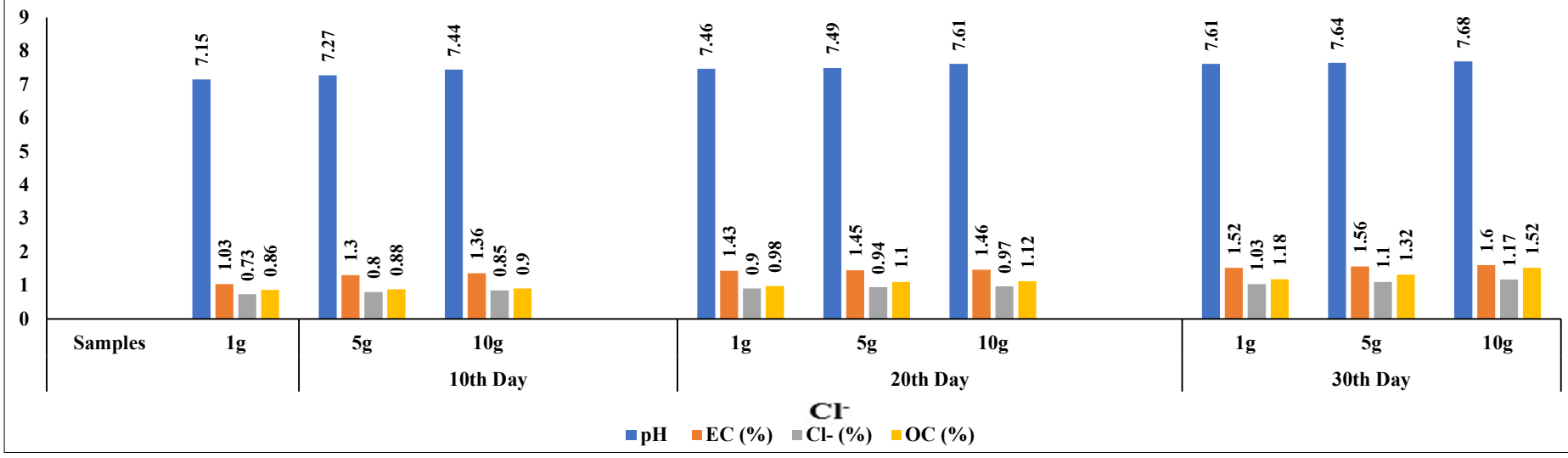


Figure 4: Graphical Representation of chemical parameters such as Ca^{2+}, Mg^{2+}, N^+, K^+, P, SO_4^{2-} of landfill site soil sample treated with expired clonozepam medicines with different concentrations at an interval of 10^{th}, 20^{th} and 30^{th} day

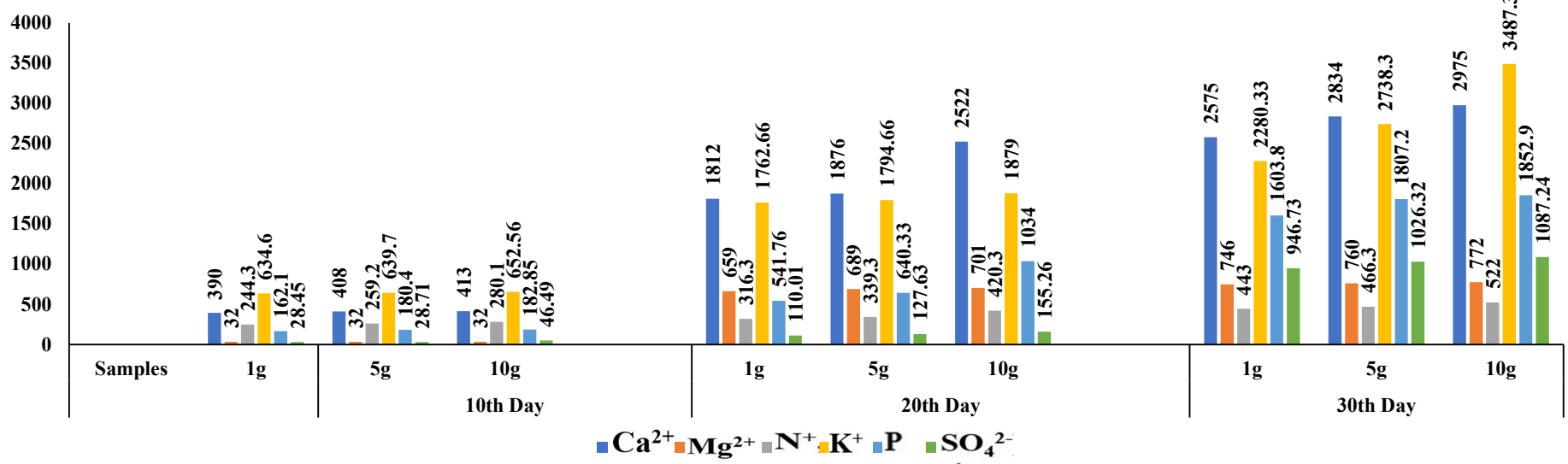
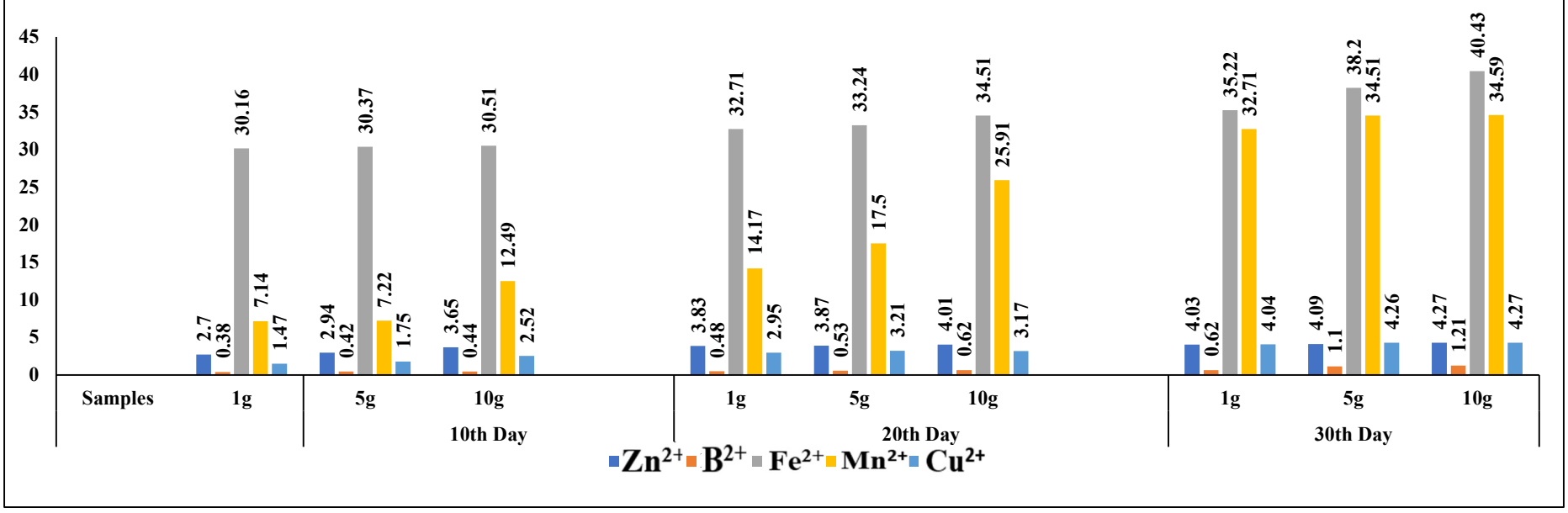


Figure 5: Graphical Representation of chemical parameters such as Zn^{2+}, B^{2+}, Fe^{2+}, Mn^{2+}, SO_4^{2-} of landfill site soil sample treated with expired clonozepam medicines with different concentrations at an interval of 10^{th}, 20^{th} and 30^{th} day



The following **Tables 1 and 2** represents the experimental findings from the evaluation of the physico-chemical parameters of soil samples treated with different concentrations of expired clonazepam tablets with soil quality standards. Every 10 days, the samples of the soil were taken from the treatments and subjected to extraction. **Table 3** represents the physico-chemical properties of soil without any addition of an expired tablet (control). From the **Figure 2-5** represents the physico-chemical parameters of landfill site soil treated with the expired clonazepam tablet with different concentrations at an interval of 10^{th}, 20^{th} and 30^{th} day. The experimental results are presented as follows.

Figure 1 represents the physico-chemical parameters of landfill site soil before the addition of expired clonazepam tablet (Control). From the study. In control, the moisture content observed was 4.46%, water holding capacity 37.62%, bulk density 1.182 Mg/m^3, particle density 1.876 Mg/m^3 and porosity as 36.994%. pH in control was observed 7.35, electrical conductivity 1.41 ds/m, calcium 4.2 ppm, magnesium 1.6 ppm, chloride 0.53%, organic carbon as 0.66%, available nitrogen as 163 Kg/ha, potassium 215.40 Kg/ha, available phosphorus 251.40 Kg/ha, sulphate as 26.68 ppm, zinc 2.94ppm, boron 0.6ppm, iron as 46.61 ppm, manganese 10.19 ppm and copper as 3.35ppm.

Moisture Content (MC): As per the present study, it was observed that, as the number of days increased, along with the concentration of expired clonazepam, the percentage of moisture content in the soil also increased. The higher moisture content 7.136 % was observed in 10g at 30^{th} day interval. Additionally, the study compares the observed moisture content with soil standards and shows that, the moisture content in the soil with expired clonazepam was higher than the standard range.

Water Holding Capacity (WHC): In the present study, the highest water holding capacity was observed in 10g concentration at 30^{th} day interval. Water holding capacity refers to the ability of the soil to retain water. In this case, the presence of the expired drug is associated with an increase in water holding capacity.

Bulk Density (BD): The Bulk density value obtained from the study was found to be increase as the number of days increase with increase in the concentration of the expired tablet. Bulk density refers to the mass of soil per unit volume and it is an important indicator of soil compaction. Specifically, in the present study shows that, the lowest bulk density (1.188 Mg/m^3) was found at the 10^{th} day with a concentration of 1g, and the highest bulk density (1.291 Mg/m^3) was observed at the 30^{th} day interval with a concentration of 10g. The study also compares these bulk density values with soil

standards and found that they fall within acceptable limits.

Particle Density (PD): In the experimental study, the highest particle density (PD) observed was 2.49 Mg/m³ at the 30th day interval. Particle density refers to the mass of soil particles per unit volume, and it is a measure of how tightly the soil particles are packed. The study compares with the observed particle density with soil quality standards for particle density, showing that the observed value (2.49 Mg/m³) falls within the permissible limits defined by the standards (2-2.65 Mg/m³).

Porosity: Porosity refers to the volume of void space in the soil and is an important indicator of soil structure and aeration. Specifically, the lowest porosity observed was 38.196 Mg/m³ at the 10th day interval with a concentration of 1g, while the highest porosity value was 48.143 Mg/m³ at the 30th day interval with a concentration of 10g. These variations in porosity indicates the changes in the soil structure over time and with different concentrations of the expired clonazepam.

pH: In the experiment, the pH content of the soil ranged from 7.15 to 7.68. pH is a measure of the acidity or alkalinity of a substance, and in the context of soil, it can influence nutrient availability and microbial activity. The pH values were found to be higher in comparison with the soil quality standard. The presence of the expired drug, clonazepam, is attributed to the higher pH values. The highest pH was observed in the 10g concentration at the 30th day interval.

Electrical Conductivity (EC): From the study, it was observed that, as the number of days increased, along with the concentration of expired clonazepam, the EC in the soil also increased. The highest EC value, 1.60 ds/m, was found in the 10g concentration at the 30th day interval.

Calcium (Ca^{2+}): The calcium (Ca^{2+}) content was found to be very high when compared with the soil standards. The highest Ca^{2+} (2957 ppm) content was observed in 10g concentration at 30th day interval. In comparison with the soil quality standard (700-36000 ppm), the Ca^{2+} content was found to be less (389.66 ppm) in 10th day interval. The Ca^{2+} content got increased in 20th and 30th day interval. Higher calcium content in soils can indeed increase pH, potentially making other nutrients less available.

Magnesium (Mg^{2+}): In the present study, the magnesium content in the soil varied over time. Specifically, the Mg^{2+} content was observed to be 32 ppm at the 10th day interval and increased to 772 ppm at the 30th day interval. In comparison with soil standards, the values obtained in the study were found to be within the permissible limits.

Chloride (Cl^-): From the study, it was observed that, there was a gradual increase

in chloride concentration with the increase in the numbers of days and the concentration of the expired medicine in comparison with the soil quality standards. The lowest percent of chloride observed was 0.737% at 10th day interval at 1g concentration. The highest percent of chloride was found to be 1.171% at 30th day interval at 10g concentration.

Organic Carbon (OC): From the study, it was found that, the percent of organic carbon in the soil was found to be higher in all the treatments compared to the soil quality standard. This is a positive observation as higher levels of organic carbon in the soil are generally beneficial for soil health and environmental sustainability. The highest organic carbon content, 1.52%, was observed in the 10g concentration at the 30th day interval.

Nitrogen (N^+): In the present study, after the addition of expired clonazepam, the nitrogen values in the soil increased with both the number of days and the concentration. The highest available nitrogen value observed was 521.66 Kg/ha at the 30th day in the 10g concentration. In comparison with the soil quality standard for nitrogen content (which ranges from 240-480 Kg/ha), it shows that the highest observed nitrogen value exceeds the upper limit of the standard. This indicates the elevation of nitrogen level in the soil due to the presence of the expired clonazepam. Excess nitrogen in the soil can leach into groundwater or runoff into surface water, contributing to water pollution and ecosystem disruption.

Potassium (K^+): From the study, in comparison with the soil quality standard, the potassium content in the soil increased with the number of days and drug concentration. The highest observed potassium content was 3487.33 Kg/ha during the 30th day interval at a concentration of 10g, while the lowest range, 244.33 Kg/ha, was obtained at the 10th day interval with a concentration of 1g. Excessive potassium levels in the soil can lead to nutrient imbalances.

Available Phosphorus (P): According to the present study, the available phosphorus in the soil increased with both the number of days and the concentration of the expired drug (clonazepam). The highest observed available phosphorus was 1852.9 Kg/ha at the 30th day interval with a concentration of 10g. In comparison with the soil quality standards, the phosphorus values were found to be higher. High phosphorus levels may contribute to imbalances in nutrient cycling.

Sulphate (SO_4^{2-}): From the study the sulphate content in the soil was within the limit at the 10th day interval with 1g and 5g concentrations of the expired clonazepam tablet. However, at the 20th and 30th day intervals, with concentrations of 5g and 10g

in the treatments, the sulphate values were found to gradually increase in comparison with the soil quality standard. The highest sulphate observed from the study was 1087.34 ppm in the 10g concentration at the 30th day interval.

Zinc (Zn^{2+}): From the experimental study, the zinc values in the soil increased as the number of days increased, along with an increase in the concentration of the expired clonazepam tablet. Specifically, the value of zinc observed at 1g concentration at the 10th day interval was 2.7 ppm. This trend indicates that, the zinc values were found to be higher with the increase in both the number of days and drug concentration. Furthermore, the highest zinc values were recorded at the 30th day interval with a 10g drug concentration, reaching 4.27 ppm.

Boron (B^{2+}): In the present study, the boron values were found to be lower compared to the soil quality standard until the 20th day interval with a 5g drug concentration. However, at the 30th day interval with a 10g drug concentration, the highest boron values were observed, reaching 1.21 ppm. Boron is a micronutrient, and its concentration in the soil needs to be within an optimal range for plant health. It's important to monitor and manage boron levels to prevent adverse effects on plant growth and to avoid potential environmental contamination.

Iron (Fe^{2+}): From the study**,** after adding the expired drug, the iron content in the soil was found to be higher compared to the soil quality standard. The maximum iron concentration observed in the study was 40.43 ppm at the 30th day interval with a 10g concentration of the expired drug.

Manganese (Mn^{2+}): From the experiment**,** the manganese values in the soil increased with both the number of days and the concentration of the expired medicine. The lowest manganese value was observed at the 10th day interval with a 1g concentration of the drug, measuring 7.146 ppm. The highest manganese value was observed at the 30th day interval, reaching 34.59 ppm. Comparing these values with the soil quality standard, it's observed that, the manganese values were found to be higher. Excessive manganese levels can lead to manganese toxicity, negatively impacting plant growth and development.

Copper (Cu^{2+}): From the study, in comparison with the soil quality standard, the copper values in the treatments were found to be higher, specifically measuring 1.477 ppm at the 10th day interval in 1g concentration of expired drug. However, the highest copper value was observed in the 10g concentration at the 30th day interval. Excess copper levels in soil can potentially have an impact on the surrounding environment.

Table 4: Organoleptic Characterization of expired clonazepam tablet		
	Characteristics	Details of Expired tablet
1	Name of the tablet	Clonazepam
2	Layer	Single layer, uncoated
3	Drug content	Clonazepam, Excipients Q. S
4	Dosage	0.5mg
5	Colour	Pale yellow/ lake of tartrazine
6	Odour	Faint odour
7	Taste	Slight bitter
8	Size	3mm diameter
9	Weight	Each tablet contains 0.131g
10	Therapeutic class	Benzodiazepines/ anticonvulsant
11	Hardness test	1.536g
12	Friability	0.0022%
13	Disintegration	6 minutes
14	Dissolution	Dissolution of drug is 0.18% at 120mins

Table 4 represents the organoleptic characteristics of expired clonazepam tablet. In the present study, clonazepam tablets belong to the therapeutic class of anticonvulsant drug. From the study, it was observed that, the layer of the drug is single layer and uncoated. The colour of the expired drug is pale yellow/ lake of tartrazine in colour. The tablets have faint smell and slight salty and bitter in tase. The size of the tablet is 3mm in diameter. The expired clonazepam drug weigh about 0.131g.

The hardness of the tablet is measured in terms of load/pressure required to crush tablets when placed on its edges. The hardness test is performed using Monsanto or Pfizer type testers. In the present study, the hardness test is used to determine the structural integrity and braking point of the tablet and to find out the changes during the storage condition, transportation, packaging and handling before usage. Hardness may affect dissolution, disintegration and bioavailability. From the experiment, the hardness of expired drug is 1.536g.

Friability tests generally refers to loss in weight of tablets in the container due to the removal of fine particles from their surfaces. It reflects poor condition of cohesion of tablet ingredients. The chamber of the friability test apparatus rotates at a rate of 25 ppm and drops the tablet from a height of 15cm. The tablets which are pre-weighed are placed in the apparatus which is given 100 revolutions after which the tablets are once again weighed. From the study, the friability of expired drug is 0.0022%. Excessive friability can compromise the uniformity of dosage. Variability in tablet strength may impact the release of the active pharmaceutical ingredient which will affect on the soil physical properties.

The disintegration test determines whether tablets disintegrate within the prescribed time when placed in liquid medium under the experimental conditions. The disintegration of a tablet refers to the process

by which the tablet breaks down into smaller particles or dissolves when exposed to physiological fluids. From the study, it was found that, the time taken by the expired drug to disintegrate is 6 minutes.

Dissolution is a test which is used to evaluate the rate of release of a drug substance from dosage form. Dissolution is the process by which a solid substance enters into a liquid known as dissolution medium or solvent to form a solution. The dissolution of a drug is important for its bioavailability and therapeutic effectiveness. In the present study, the dissolution medium was maintained at 37ºC with a rotation speed of 50rpm. The quantification of drug released from the dissolution study, was conducted by UV spectrophotometer at 254nm. From the present study, it was observed that, the percentage of clonazepam release was 0.18% within 2hours duration

Comparison of the organoleptic properties of the expired clonazepam with the soil quality parameters:

In order to study the impact of expired clonazepam tablet, the organoleptic properties of the expired tablet were studied along with the chemical composition of the expired clonazepam tablet. In the present study, the organoleptic properties like drug content, odour, taste, size, weight, hardness, friability, disintegration and dissolution test were conducted. In order to know the influence of expired tablet on soil quality, the chemical composition was observed which includes $C_{15}H_{10}ClN_3O_3$; 5-(2-Chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepin-2-one. In addition, each tablet contains the following inactive ingredients: docusate sodium, lactose monohydrate, magnesium stearate, microcrystalline cellulose, sodium benzoate, and sodium starch glycolate.

From the hardness, friability, disintegration and dissolution test, it showed that, the expired clonazepam tablet showed 0.0022% of hardness which indicates the drug can easily disintegrate or break quickly once reaches to any external environmental conditions. The disintegration and dissolution test showed that, the drug gets 6 minutes to disintegrate and 0.18% of dissolution at 120 minutes. This clearly implies that within 2 hours it can effectively dissolve once it comes in contact with moisture. The landfill since it is frequently loaded with other category of wastes due to the decomposition, moisture level will rise in the waste, this in turn help to dissolve any kind of the tablet that might enter into the landfills. This has been confirmed by the other researches also. From the observation of chemical composition showed the presence of nitrogen, magnesium, chloride, potassium and organic carbon upon disintegration all these compounds will release to the soil environment. This was

confirmed with the present pilot study. The results obtained clearly indicates that, the expired clonazepam drug will influence over the soil property if its concentration reaches to higher level.

IV. SUMMARY AND CONCLUSION:

Based on the experiment conducted with the amendment of expired clonazepam medicine into the landfill soil sample, the physical parameters such as moisture content, water holding capacity, bulk density, particle density were found to be higher when compared to control. But porosity values obtained were found to be within the range. From the experiment, it was found that, the chemical parameter such as calcium, magnesium, available nitrogen, potassium, available phosphorus and sulphate values were found to be very much higher in comparison with the soil before addition of expired medicine. Due to this, the soil near landfills may get contaminated, which will cause leachate to seep through the soil profile and cause damage to the upper part of the soil, reduction in soil quality and hurting plant life due to the presence of pharmaceuticals. From the organoleptic characterization of expired clonazepam tablet showed the influence over the soil property when its concentration reaches to higher level. There will be long-lasting consequences on the environment because of the presence of xenobiotic substances (such as benzene, hydrocarbons, phenolic compounds, etc.), including toxicity and biological accumulation in organisms' cells. These xenobiotic organic substances can get into agricultural soils, surface water, or groundwater directly. These substances will have an impact on the aquatic ecosystem. Additionally, these substances have the potential to enter the human food chain and harm people in different ways. From the present research, the tablets are not intended to be disposed of directly in soil. The recommended methods for pharmaceutical disposal involve specific guidelines, such as, using pharmaceutical take-back programs, where the medications are collected and disposed of in an environmentally responsible manner.

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