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ORIGINAL ARTICLE

Physiochemical Profiling and Spectrophotometric Assessment of Distillery Stillage

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Abstract

Objective: To investigate the physicochemical and spectrophotometric parameters, presence of heavy metals and ions, microbiota population in the distillery stillage, on soil samples collected from two sites (direct outlet and in the vicinity of discharged sites) of Ashoka Distillers and chemicals, Hathin, Palwal district, Haryana, during July 2023. **Methods:** Stillage and soil sample collected from direct discharged sites and vicinity area to study physicochemical parameters pH, electrolytic conductivity (EC), total dissolved salts (TDS), total suspended solids (TSS), biological oxygen demand (BOD), chemical oxygen demand (COD), heavy metals and ions, spectrophotometric parameters (refractory pollutants), grease or oil particles, functional groups and impact on soil. **Findings:** Study revealed high BOD (38,321$\pm$48 and 28,152$\pm$37 mg/L), COD (62,346$\pm$112 and 50,000$\pm$89 mg/L), TDS (3500$\pm$279 and 2820$\pm$258 mg/L), TSS (42,240$\pm$378 and 39,900$\pm$355 mg/L), TOC (56,400$\pm$234 and 38,001$\pm$167 mg/L), TN (4,350$\pm$120 and 2,314$\pm$78 mg/L), pH (3.2$\pm$0.10 and 3.64$\pm$0.14), EC (5.40$\pm$1.45 and 4.80$\pm$1.2), were found to be statistically different. Mercury (0.20 and 0.05 mg/L), Lead (0.9 and 0.02 mg/L), Arsenic (0.4 and 0.02 mg/L), Iron (4.82 and 3.42 mg/L), Copper (0.76 and 0.45 mg/L), Manganese (3.42 and 2.80 mg/L), Zinc (3.24 and 2.76 mg/L), Nickel (17 and 2.48 mg/L) and chloride ions (2,819 and 1876 mg/L) were also observed. Various refractory pollutants, 2,4' DDD, 2,4' DDE, DDT, Alachlor, Aldrin, Endosulfan, Altrazine, Butachlor, Beta HCH, Dieldrin, Endosulfan, sulphate, Ethion, Malathion etc., were concluded, but no grease or oil particles were found statistically different in the samples collected from two different sites. Presence of bacterial (Coliform) and fungal strains (yeast) was detected, and the soil where effluents were discharged showed different physical properties (pH, EC, organic matter, phosphate, chloride, sulphate). All the parameters were found to be more than the permissible limits except the refractory pollutants, which were found to fall within the permissible limits of the United States Environmental Protection Agency (USEPA) and the Central Pollution Control Board (CPCB). **Novelty:** The present study aimed to mitigate detrimental stillage disposal implications on soil structure (salinization, acidification), water quality deterioration (evident through increased BOD and COD levels), and accumulation of pesticide pollutants, heavy metals, and ions in both the stillage samples and contaminated soil utilizing cereal molasses. This study aims to provide evidence of the toxicity caused by stillage in the nearby area of the discharged units.

Keywords: Distillery stillage; Contamination; Refractory pollutants; Effluents; Permissible limits

1 Introduction

India is the third largest producer of alcohol by generating 3.25 billion liters of alcohol per annum, approximately in nearly 319 distillery industries in local, tropical, and subtropical regions[1]. Alcohol production can be achieved by using sugars, cereals, fruits, and other resources, and the industry dealing is known as the Distillery Industry. The stillage (residual liquid byproduct from alcohol production, particularly in distilleries) poses a significant environmental threat. The composition of distillery stillage varies depending on the raw material and fermentation process used. In this study, chemical analysis of stillage obtained from the cereal molasses alcohol industry was done. The high organic load, chemical content, and acidic nature of stillage can lead to water pollution, soil degradation, and harm to aquatic and terrestrial organisms[2]. Evidence from the literature shows the harmful impact of stillage on soil, water, microbes, and aquatic and terrestrial organisms. The microbial analysis provides insights into the composition and diversity of the microbial community, which can inform treatment strategies and enhance the understanding of the biodegradation process occurring within stillage. Effective treatment and disposal methods are crucial to mitigate its impact on the environment and living organisms[3],[4]. Several studies conducted on the characterization and analysis of stillage samples collected from different industries have revealed a huge bulk of pollutants in it. The stillage is known to contain variable range of pesticides and refractory pollutants some of them are mutagenic and endocrine hormone disrupters pollutants like butane dioic acid, 2 hydroxy isocaproic acid, vanillyl propionic acid, hexadecenoic acid, bis(trimethylsilyl) ester; hexadecane; 2,6,11,15- tetra-methyl dodecane; 2,6,10 trimethyl stigma sterol and Sitosterol trimethylsilyl ether were also observed[2]. These refractory pollutants (pesticide residues) contribute to BOD (Biochemical Oxygen Demand) and COD (Chemical Oxygen Demand) loads in the aquatic bodies when introduced without any prior treatment. Distillery wastewater had elevated levels of total solids (TS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD), as well as various heavy metals and refractory pollutants[1],[5]. Besides the heavy metals, some trace elements were also observed[6]. The evaluation of the various parameters of stillage must be done before addition to the environment[7]. The amount of production of the stillage is a hazard, and hence, a strategy must be incorporated, rationalizing its impact on nature[8],[9]. The present study aims for the complete analysis of the collected stillage sample employing cereal molasses from different sites of collection, directly from the outlet, and a place in the vicinity to draw an index of pollutants. Cereals are rich in dietary fiber and contain significant amounts of beta-glucan. This study includes profiling of microorganisms inhabiting the stillage. The profiling allows for the development of various treatment techniques aimed at effectively processing stillage. The dumping of untreated stillage in open environments toxifies the aquatic bodies, impacting the agriculture and terrestrial biota. Further investigation conducted to assess the impacts of stillage on the soil also disclosed the physicochemical conditions of the affected soil. However, studies dealing with the cons of stillage from the cereal molasses alcohol industry are lacking. Therefore, a thorough evaluation of the presence of heavy metals, metals, pesticides, and pollutants was done on cereal molasses stillage in this study. The present study was executed to fill this research gap, as there was a significant variation in the characteristics of the samples collected from the two different sites, indicating spatial heterogeneity in contamination levels and environmental impact. Similarly, the analysis of collected soil samples reflected consistent patterns of variation, further supporting the observed differences between the sampling sites. The presence of various pollutants in the samples poses serious risks to the living organisms, as these contaminants can enter the food chain and exert harmful effects. Therefore, it is essential to develop and implement an efficient, cost-effective, and environmentally friendly remediation strategy to mitigate their impact.

2 Methodology

2.1 Sample Collection

The sample was collected from the discharged unit of the industry Ashoka Distillers and chemicals located in Palwal district (28.15·N77.33·E), Haryana, India. The stillage and soil samples were collected from the area near the outlet of stillage (sample 1) in pre-sterilized containers, and a sample was collected from the vicinity of discharged units (sample 2)[2],[6]. All experiments were conducted in triplicate to ensure the reliability and reproducibility of the results. The sample was stored in the refrigerator at 4°C to prevent microbial activity.

2.2 Physicochemical and spectrophotometric analysis

The sample was qualitatively analyzed for various parameters like pH (Eutech cyber scan pH2100 pH meter), Electrical conductivity and TDS (Labman LMCM20 instrument), TSS (standard methods), BOD & COD (Titration methods), Total organic carbon (TOC), Total Nitrogen (TN) by following the methods under the protocol of APHA-AWWA-WPCF (American Public Health Commission, American Water Works Association, Water Pollution Control Federation, 2017). Refractory pollutants remain in nature as such and are non-biodegradable in nature and were analyzed through the technique LC-MS

(Liquid Chromatography-mass spectrometry). ICP-MS (Inductively Coupled Plasma Mass Spectrometry) was used to analyze ions, heavy metals, and trace elements. FT-IR (Fourier transform-infrared spectroscopy) was used to check the presence of grease or oil, and the functional group of various compounds present in the stillage. Micro-floral gel plate count technique was used to reveal the microorganisms inhabiting the stillage. The potent refractory pollutants are major pesticide residues; hence, their impact on pest termites is also studied here by exposing them to different concentrations of stillage at different periods.

2.3 Statistical Analysis

Comparison of various parameters collected from different sites, the mean ± standard deviation was calculated for the collected data from the different sites in replicates. The differences in data were tested by using one-way ANOVA at a level of significance $P \leq 0.05$ using SPSS Software (IBM SPSS, Version 29.0.2.0).

3 Results and Discussion

The detailed analysis of the sample discusses physicochemical properties based on various parameters like pH, Odor, TDS, TSS, BOD & COD (Table 1) of the stillage sample and the impact on soil (Table 2). Some parameters were within permissible discharge limits, while others exceeded the limits, indicating the sample's potential toxic effects on the environment. The physicochemical analysis indicated the presence of several heavy metals and ions as listed in Table 1 by using the ICP-MS technique.

Table 1. Physicochemical properties of the stillage sample and metals and ions found

Sr. no.	Parameters	Analyzed Value Sample 1 (Discharged site)	Analyzed Value Sample 2 (Site in vicinity of outlet)	Permissible limits USEPA	Permissible limits CPCB
1.	Color	Dark Brown	Brown	—-	—
2.	Odor	Pungent	Pungent	—-	—
3.	pH	3.2±0.10	3.64±0.14	5-9	5.5-9
4.	Electrical Conductivity (siemens/cm)	5.40±1.45	4.80±1.2	—	0.00225
5.	TDS (mg/L)	3500±279	2820±258	2100	2100
6.	TSS (mg/L)	42,240±378	39,900±355	—	—
7.	BOD (mg/L)	38,321±48	28,152±37	40.00	Less than 10 mg/L
8.	COD (mg/L)	62,346±112	50,000±89	120.00	100
9.	TOC (mg/L)	56,400±234	38,001±167	—–	—-
10	TN (mg/L)	4350±120	2,314±78	—-	—-
11	Mercury Hg (mg/L)	0.20±0.00	0.005±0.00	0.002	0.01
12	Lead Pb (mg/L)	0.9±0.00	0.02±0.00	0.05	0.05
13.	Arsenic As (mg/L)	0.4±0.01	0.02±0.00	0.010	0.01
14.	Iron Fe (mg/L)	4.82±0.05	3.42±0.00	2.0	0.3
15.	Copper Cu (mg/L)	0.76±1.1	0.45±0.00	0.050	0.05
16.	Manganese Mn (mg/L)	3.42±0.02	2.80±0.00	2.0	—
17.	Zinc Zn (mg/L)	3.24±0.05	2.76±0.00	2.0	0.1
18.	Nickel Ni (mg/L)	17.0±2.1	2.48±0.00	0.10	0.1
19.	Chloride Cl^{-} (mg/L)	2819±3.2	1876±0.00	1500	1000

However, some ions were also detected in elevated amounts in soil samples 1 and 2, as depicted in Table 2, as compared to standard values. In other studies, elevated levels of pollutants were also concluded in the soil near the discharged sites[6].

Table 2. Physicochemical aspects of soil samples

S. No.	Physical parameter	Sample 1	Sample 2	Permissible limits under ISO 28258
1.	pH	6.2±0.14	7.68±0.21	6.0-7.5
2.	Electrical Conductivity (mS/cm)	4.4±0.15	2.14±0.18	Less than 4.0
3.	Organic Matter (%)	18.26±2.4	12.78±0.98	10-12%
4.	Phosphate (mg/L)	753±2.1	244±3.4	11
5.	Chloride (mg/L)	1278±3.8	843±2.9	—
6.	Sulphate (mg/L)	122±.19	78±1.2	—

The FT-IR technique was employed to identify the presence of mineral oils, absorption spectra, and functional groups within the sample. The chromatogram obtained from the FT-IR investigations revealed no evidence of grease or mineral oils in the sample, but various kinds of persistent pollutants showed different absorption peaks as shown in the chromatogram (Figure 1).

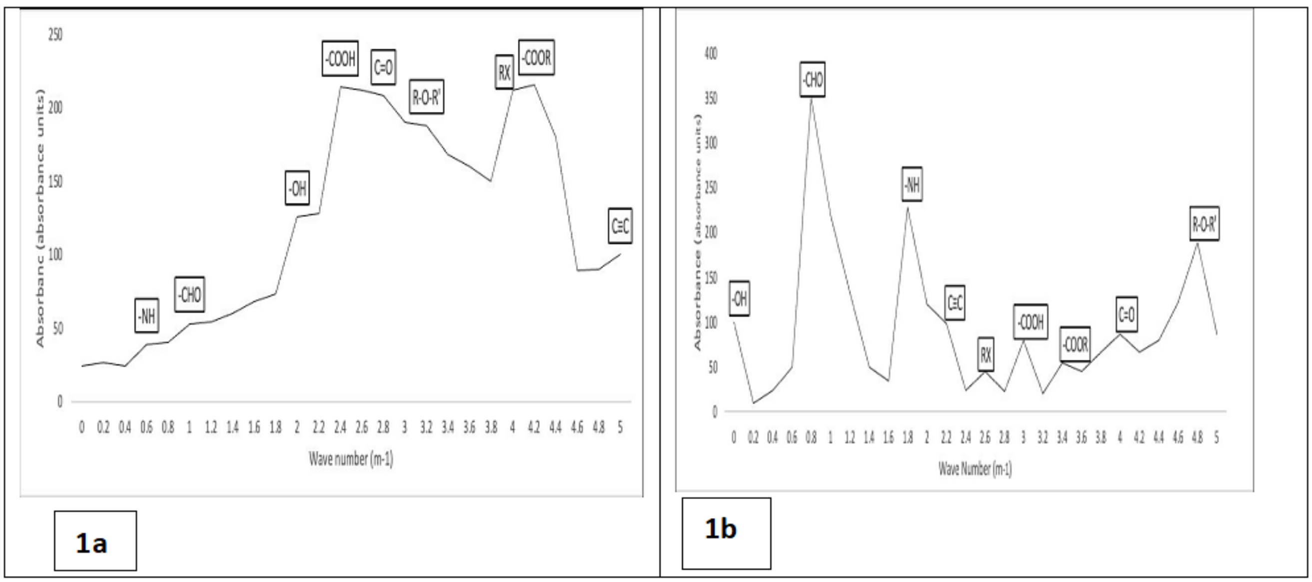

Fig 1. The chromatogram produced during FT-IR

Discussion:

Conducting a thorough analysis of stillage is essential before its introduction into the environment, as it provides a comprehensive assessment of both organic and inorganic components. The physicochemical analysis conducted on the sample quantified the organic load being contributed to the environment[8]. Spectrophotometric experiments revealed the presence of organic waste and molecules resembling pesticides. The sample had an acidic pH exceeding permissible limits, which may lower the pH of the surrounding environment, as studies on dry stillage (rye stillage) with low pH suggest minimal impact on soil pH, though it increases electrolytic conductivity[10]. The associated ions can disrupt environmental properties and adversely affect local organisms like planktons; fish, prawns grow in a specific pH will die due to a low pH. The sample's electrical conductivity exceeded permissible limits, indicating high ion presence. The ions present include bicarbonates, carbonates, and hydroxides, which contribute to increased EC. In this analysis, only a few base ions were observed, leading to variability in EC measurements reported by different authors. TDS, TSS, BOD & COD serve as essential indicators reflecting the organic pollution load in aquatic ecosystems. Elevated concentrations of these parameters can promote biomagnification, whereby pollutants progressively accumulate across higher trophic levels. Excessive organic matter fosters eutrophication, stimulating microbial proliferation to aerobically degrade these substances. This process depletes dissolved oxygen, potentially resulting in the mortality of aquatic organisms such as fish. Furthermore, oxygen deficiency can disrupt key physiological parameters, including glucose, glycogen, and lactic acid levels in affected species[11]. Elevated levels of these parameters exceeded permissible limits, increasing the organic load in the affected water body. Different samples identified by different

Table 3. LC-MS-based assessment of refractory pollutants

S.NO	Refractory pollutants	Impact on Natural Communities	Reference
1.	2,4’ DDD (dichlorodiphenyldichloroethane)	Thyroid disturbance, eggshell thinning, bioaccumulation, and toxicity in aquatic biota	Stockholm Convention (2001)
2.	2,4’DDE (dichlorodiphenyldichloroethylene)	Cancer, Diabetes, Eggshell thinning, Cancer	Stockholm Convention (2001)
3.	2,4’DDT (dichlorodiphenyldichloroethane)	Impairment of the nervous system, Kidney, Liver	Stockholm Convention (2001)
4.	2,4-Dichlorophenoxy Acetic Acid	Skin and Eye irritation, cancer, vascular disease, decreased growth rates	EPA (2012)
5.	4,4’ DDD (dichlorodiphenyldichloroethane)	Thyroid disturbance, eggshell thinning, bioaccumulation, and toxicity in aquatic biota	Stockholm Convention (2001)
6.	4,4’ DDE (dichlorodiphenyldichloroethylene)	Cancer, Diabetes, Eggshell thinning, Cancer	Stockholm Convention (2001)
7.	4,4’ DDT (dichlorodiphenyldichloroethane)	Impairment of the nervous system, Kidney, Liver	Stockholm Convention (2001)
8.	Alachlor, Butachlor	Potent herbicide	USEPA (2012)
9.	Aldrin	Potent pesticide and an inhabitant of termites	Stockholm Convention (2001)
10.	Alpha and Beta endosulfan	Lethal to pests	Stockholm Convention (2001)
11.	Alpha HCH, Beta HCH, Gamma-HCH, and Delta HCH (Hexachlorocyclohexane)	Toxic to birds, mammals, and aquatic organisms	Stockholm Convention (2001)
12.	Atrazine	Weedicides, nausea, and irritation in exposed parts.	Stockholm Convention (2001)
13.	Lactic acid DITMS	Irritation of the skin	—-
14.	Dieldrin	Refractory in nature and a potent pesticide	Stockholm Convention (2001)
15	Endosulfan sulphate	Skin and eye irritation, drowsiness, accumulation in the gonads and liver of fish	Stockholm Convention (2001)
16.	Ethion	Nausea, cramps, blurred vision, headache	Stockholm Convention (2001)
17.	Silane	—-	—-
18.	Stigmasterol	—-	—-
19.	Eicosane	Antifungal	—–
20.	Pentachloro-bromobenzene	Endocrine-disrupting compounds	—-
21.	Hexadecane	Nervous system breakdown, Gastrointestinal irritation	USEPA 2012

Table 4. The micro-biota of the sample

S. No.	Test Parameters	Result
1.	Total plate count	3.95×10^4 cfu/g
2.	Total yeast and mold count	350 cfu/g
3.	Coliform count	10 cfu/g.

authors revealed varying constituents with different functional groups, resulting in distinct chromatographic peaks. The FT-IR technique revealed a chromatogram depicting UV light rays' absorption by the sample's components, with each peak indicating a specific compound's absorbance[8]. LC-MS spectroscopy revealed numerous refractory pollutants that are persistent and non-biodegradable (Table 3). These compounds accumulate in ecosystems, entering food chains and causing adverse effects such as diseases and inhibited growth in animals and plants. These pollutants share a chemical similarity with pesticide residues and are non-degradable. Although present in limited quantities within permissible limits, their persistence poses a toxic threat to the environment due to their inability to break down. Various studies using LC-MS techniques have revealed that different samples contain diverse types of products, as reported by multiple authors[12]. The microfloral count revealed the presence of various microscopic species in the collected sample, contributing to its natural degradation (Table 4). In addition to bacterial strains, fungal strains were also observed[13]. Thus, from the above discussion, it is evident that treatment techniques must comply with environmental regulations to prevent toxic contamination. Even the byproducts of the stillage can be used for Methane production, which serves as a valuable biofuel[14]. A study revealed that the stillage contains chemicals that can serve as precursors or substrates for the production of other compounds[15]. Stillage is rich in organic compounds, which can be converted into fertilizers as it has some heavy metals as well[6],[16]. The most popular step these days involves valorization of the stillage sample[17]. Even wet air oxidation (chemical process which uses water and air to break down pollutants that are too solid for other treatments, like digestion. Traditional methods use high temperatures and pressures, while advanced methods use even higher temperatures and pressures. Although the technology is well developed, researchers are still working to make it more efficient and affordable, mainly by using catalysts. Wet air oxidation with catalysts is seen as an eco-friendly and cost-effective option[18]. Recent research focuses on innovative, eco-friendly, and cost-effective ways, such as plant-microbe technologies, to treat and recycle distillery waste[19].

4 Conclusion

The stillage generated from distillery processes contains several harmful pollutants contributing to the elevation of toxic substances. This study aimed to explore the significant difference in the pollutants received from the two different sites of an industry, but the refractory pollutants were very less in count, but they were nondegradable and persistent. It was revealed that the sample was highly toxic and hazardous to the environment. Even the physicochemical properties of the soil sample were changed, leading to unproductive soil. The failure of crop production in such areas will affect the economy of the local people. Hence, good techniques should be accepted to prevent the hazardous impact of pollutants on the environment. Future recommendations include scaling up lab-scale technologies to industrial applications for processing the stillage. Long-term monitoring should be conducted to assess environmental safety, and cost-benefit analysis should be explored for implementation.Non-Cited References[20],[21]

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