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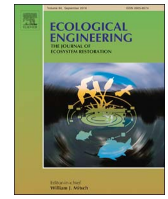

Heavy metal phytoextraction potential of native weeds and grasses from endocrine-disrupting chemicals rich complex distillery sludge and their histological observations during in-situ phytoremediation

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ABSTRACT

Sugarcane-molasses based distillery waste is a threat to environment for its safe disposal due to complexation of endocrine-disrupting chemicals (EDCs) containing mixture of organic pollutants. This study revealed that distillery sludge contains not only mixture of complex organic pollutants but also retains high quantity of Fe (5264.49), Zn (43.47), Cu (847.46), Mn (238.47), Ni (15.60), and Pb (31.22 $mg\ kg^{-1}$) which enhances the toxicity of sludge to the environment. The major identified organic compounds were benzene, 1-ethyl-2-methyl, benzene, 1-ethyl-4-methyl benzoic acid, 3,4,5-tris(TMS oxy), TMS ester; hexanedioic acid, dioctyl ester; stigmasterol TMS ether; 5α-cholestane,4-methylene; campesterol TMS; β-sitosterol and lanosterol. These compounds are listed under the EDCs also as per U.S. Environmental Protection Agency. However, the phytoextraction potential of growing native weeds and grasses i.e. *Argemone mexicana, Saccharum munja, Cynodon dactylon, Pennisetum purpureum, Chenopodium album, Rumex dentatus, Tinospora cordifolia, Calotropis procera* and *Basella alba* revealed the high accumulation of Fe, Zn, Cu, Mn, Ni, and Pb in their root and leaves compared to shoot. This indicated high accumulation and translocation capabilities of these plants. Further, the bioaccumulation coefficient factor (BCF) and translocation factor (TF) was found > 1 for majority of plants for various metals. Thus, this given strong evidence for hyperaccumulation tendency of these native weeds and grasses from complex polluted sites. Furthermore, the ultrastructural observations of root tissues also revealed the deposition of heavy metals at various cellular components without any apparent toxic effects. This indicated the variable adaptive characteristics of these plants growing at a hazardous waste polluted site. Thus, the study given a strong evidence for application of these weeds and grasses as tools for in-situ phytoremediation and eco-restoration of polluted sites.

1. Introduction

Sugarcane-molasses based distillery waste is well known as source of complex environmental pollutants due to various heavy metals containing complex organic pollutants (Chandra et al., 2008; Chandra and Kumar, 2017a, 2017b). In India, there are more than 397 sugarcane molasses based distilleries releasing approximately 3.5×10^{13} kL spent wash annually (AIDA, 2016). There is an average sludge generation of 1500 tons per day during anaerobic digestion of spent wash (Kansal et al., 1998). This reflects the magnitude of the environmental pollution caused by the waste generated from distillery sector all over India. The sludge generated from distilleries also contain mainly dodecanoic acid, octadecanoic acid, *n*-pentadecanoic acid, hexadecanoic acid, β-sitosterol, stigmasterol, β-sitosterol trimethyl ether, heptacosane, dotriacontane, lanosta-8, 24-dien-3-one, 1-methylene-3-methyl butanol, and 1-phenyl-1-propanol as androgenic and mutagenic compounds (Chandra and Kumar, 2017a), which are listed under the endocrine-disrupting chemicals (EDCs) list of USEPA (2012). The study has revealed that these organic pollutants makes organo-metallic complex with various heavy metals which are mainly iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd), manganese (Mn), nickel (Ni), and lead (Pb) present in high quantity i.e. (Fe: 2403.64), (Zn: 210.624), (Cu: 73.63), (Cr: 21.84), (Cd: 1.446), (Mn: 126.292), (Ni: 13.425), (Pb: 16.332 $mg\ kg^{-1}$) (Chandra and Kumar, 2017c). The concentrations of these metals are for above than the prescribed limit in environment as per USEPA (2002) and European Union (2002).

Generated effluent after distillation process also a major source of aquatic pollution due to high level of maillard products generated due

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to complexation of sugar and amino acid at elevated temperature (Chandra and Kumar, 2017c). It has been reported that melanoidins have net negative charges, hence, different heavy metals (Cu^{2+}, Cr^{3+}, Fe^{3+}, Zn^{2+} and Pb^{2+} etc.) strongly bind to form organo-metallic complex with melanoidins (Migo et al., 1997). The high metal binding tendency of melanoidins also enhances vulnerability of organo-metallic complex towards its toxicity in environment. Consequently, the sludge generated after anaerobic digestion contains mixture of various complex organic pollutants along with heavy metals, phenolics, and EDCs which are generated in sugar production and molasses fermentation (Chandra and Kumar, 2017a, 2017c). The study have shown the toxicity of heavy metals at the genetic and cellular level both due to generation of reactive oxygen species under metal stress which can seriously disrupt normal metabolism of the plant and animal both (Nagajyoti et al., 2010; Jaishankar et al., 2014). Oxyradicals can cause lipids peroxidation, inactivation of enzyme and membrane damage leads to cause cellular toxicity in plants and human (Kumar et al., 2013a, 2013b; Tian et al., 2012). Consequently, the contamination of distillery waste to aquatic resources not only affects the water quality but also adversely affect to aquatic flora and fauna (Bharagava and Chandra, 2010; Ayyasamy et al., 2008). In addition, the presence of androgenic and mutagenic compounds in the industrial sludge not only changes the soil quality but also adversely affect to soil microbial communities which are essential for elemental recycling and maintenance of soil fertility (Juwarkar and Dutta, 1989). But, the recent studies have reported that to mitigate the oxidative damage, plants have developed a complex defense antioxidant system including low molecular weight antioxidants (cystein, ascorbic acid, and protein thiol) as well as enzyme such as superoxide dismutase, catalase and peroxidase (Kumar et al., 2013a, 2013b; Tian et al., 2012). Thus, these antioxidants play an important role in the cellular defense strategy of plants against oxidative stress, inducing resistance for metals by protecting labile macromolecules (Chandra and Yadav, 2010). However, the knowledge regarding the presence of androgenic and mutagenic compounds in distillery waste is still not well known to majority of the environmentalist. Therefore, the development of detoxification device of distillery waste including the sludge is essential prior to its disposal in the environment for sustainable development.

There are certain hyperaccumulator plants with characteristic properties of fast growth and production of high biomass of shoot along with diversified rhizospheric microbial communities, which facilitate the bioavailability of plant nutrient and mineralization of complex organic pollutants (Sessitsch et al., 2013; Rajkumar et al., 2012; Guo and Cutright, 2014). The bacterial assisted phytoremediation of organic and inorganic pollutants is regulate by soil texture, pH, temperature and other environmental factors which regulated the heavy metal accumulation pattern in the different parts of plant and detoxification mechanism of organic pollutants (Ma et al., 2011; Sinha et al., 2007). Though, few reports have highlighted the ability of some wetland plants to remove the heavy metals from the organic pollutants containing wastewater in natural and constructed wetland (Weis and Weis, 2004; Deng et al., 2004). A study from Kolkata (India) also showed that 10 common regional wetland plant species from a wetland site accumulated metals like Cd, Cr, Cu, Pb, Zn, Mn and Fe (Chatterjee et al., 2011). The study reported water hyacinth (*Eichhornia crassipes*), water spinach (*Ipomoea Aquatica*), watermeal (*wolffia arrhiza*), water chestnut (*Trapa bispinosa*), water lettuce (*Pistia stratiotes*), common arum (*Colocasia esculenta*), common sedge (*Cyperus rotundus*), bulrush (*Scirpus sp.),* arrowhead weed (*Sagittaria montevidensis*), bermuda grass (*Cynodon dactylon*) for heavy metal accumulation efficiency in their different parts. Further, they also reported that such plants were naturally ameliorating metal contamination from the wetland site, thus acting as sustainable and cost effective natural effluent treatment system for bioremediation. Some studies on phytoextraction potential of various plants and their histological observations have been reported globally from pure metal solution in-vitro conditions only (Maruthi Sridhar et al., 2005; Najeeb et al., 2011; Alkhatib et al., 2013; Daud et al., 2013). However, the histological observations of these plants due to heavy metal accumulation in their tissue are not revealed so far. Furthermore, the phytoextraction capabilities of native weeds and grasses growing at complex organic polluted site is not yet reported for heavy metal accumulation to explore in-situ phytoremediation potential for eco-restoration of polluted sites with complex organic waste. Moreover, the heavy metals phytoextraction potential by native weeds and grasses growing on complex organo-metallic compounds which are rich with androgenic and mutagenic compounds listed under EDC groups (USEPA, 2012).

Therefore, the present study has been focused on detail investigation of complex organic pollutants present in distillery sludge by Gas chromatography-mass spectrometry (GC–MS) analysis and microscopic histological observations of root of growing weeds and grasses by transmission electron microscope (TEM) for heavy metal accumulation in their parts to reveal the hyperaccumulation mechanism of these potential plant species in presence of complex pollutants.

2. Materials and methods

2.1. Site description

The test site of the experiments selected for soil and plant sampling was located in Unnao, Uttar Pradesh (26°320″ N, 80°30′0″E), India (Fig. 1). The samples were taken from the sludge dumping site of M/s Unnao Distilleries & Breweries. This site is well known for high pollution with organic and inorganic pollutants reported earlier by various researchers (Chandra et al., 2008; Chandra and Kumar, 2017c)

2.2. Collection of plant and distillery sludge samples

Nine representative native plants species (weeds and grasses) were collected based on dominant species luxuriantly growing on disposed distillery sludge. These plants species were identified from different genera and families according to Dutch flora of Indo-Gangetic plains, where three species *Saccharum munja* (munja), *Cynodon dactylon* (bermuda grass), and *Pennisetum purpureum* (elephant grass) belong to Gramineae family while other five plants namely *Argemone mexicana* (mexican poppy), *Chenopodium album* (goosefoots), *Rumex dentatus* (toothed dock), *Tinospora cordifolia* (giloy), *Calotropis procera* (madhaar), and *Basella alba* (pui) belonging from Papaveraceae, Amaranthaceae, Polygonaceae, Menispermaceae, Asclepiadaceae, and Basellaceae, respectively. These plants species were uprooted with associated sludge samples and carried in pre-sterilized polythene bags for the analysis of accumulated heavy metal in different parts of growing plants. Besides, the fresh disposed dried distillery sludge cakes were collected in clean pre-sterilized polythene bags from sludge dumping site of distillery plant located inside the premises of industry. The fresh as well as ameliorated distillery sludge after plant growth was collected randomly in triplicate from three different points of same location. This process was repeated three times in different seasons from same place which was protected from any outside interference of human and animals.

2.3. Physico-chemical analysis of distillery sludge

The physico-chemical parameters of distillery sludge sample i.e. pH, electrical conductivity (EC), chloride (Cl^-), sodium (Na^+), and nitrate were estimated according to the method described by Kalra and Maynard (1991). The phenol contents in sludge were analyzed as per standard methods described in APHA (2012). The pH and EC values (sludge:water = 1:2.5 w/v) of sludge samples were measured by using Orion meter (Model-960, Thermo Scientific, FL, USA) and Orion conductivity meter, respectively (Chandra et al., 2008). The total content of Fe, Zn, Cu, Mn, Ni, and Pb in dry weight sample of sludge was measured