Middlesex University Research Repository

An open access repository of

Middlesex University research

<http://eprints.mdx.ac.uk>

Tripathi, Sonam, Sharma, Pooja, Singh, Kshitij, Purchase, Diane ORCID logoORCID:
<https://orcid.org/0000-0001-8071-4385> and Chandra, Ram (2021) Translocation of heavy
metals in medicinally important herbal plants growing on complex organometallic sludge of
sugarcane molasses-based distillery waste. Environmental Technology and Innovation, 22 ,
101434. ISSN 2352-1864 [Article] (doi:10.1016/j.eti.2021.101434)

Final accepted version (with author's formatting)

This version is available at: <https://eprints.mdx.ac.uk/32248/>

Copyright:

Middlesex University Research Repository makes the University's research available electronically.

Copyright and moral rights to this work are retained by the author and/or other copyright owners unless otherwise stated. The work is supplied on the understanding that any use for commercial gain is strictly forbidden. A copy may be downloaded for personal, non-commercial, research or study without prior permission and without charge.

Works, including theses and research projects, may not be reproduced in any format or medium, or extensive quotations taken from them, or their content changed in any way, without first obtaining permission in writing from the copyright holder(s). They may not be sold or exploited commercially in any format or medium without the prior written permission of the copyright holder(s).

Full bibliographic details must be given when referring to, or quoting from full items including the author's name, the title of the work, publication details where relevant (place, publisher, date), pagination, and for theses or dissertations the awarding institution, the degree type awarded, and the date of the award.

If you believe that any material held in the repository infringes copyright law, please contact the Repository Team at Middlesex University via the following email address:

eprints@mdx.ac.uk

The item will be removed from the repository while any claim is being investigated.

See also repository copyright: re-use policy: <http://eprints.mdx.ac.uk/policies.html#copy>

Translocation of heavy metals in medicinally important herbal plants growing on complex organometallic sludge of sugarcane molasses-based distillery waste

Sonam Tripathi^a, Pooja Sharma^a, Kshitij Singh^a, Diane Purchase^b, Ram Chandra^{a,*}

^a Department of Environmental Microbiology, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar Central University, Vidya Vihar, Raebareli Road, Lucknow, Uttar Pradesh 226025, India ^b Department of Natural Sciences, School of Science and Technology, Middlesex University, The Burrough, London NW4 4BT, UK

* Corresponding author.

E-mail address: prof.chandrabbau@gmail.com (R. Chandra)

Graphical Abstract

Abstract

This study aimed to assess the heavy metals accumulation patterns by some native plants such as *Achyranthus aspera* L., *Amaranthus viridis*, *Basella alba* L., *Sesbania bispinosa*, *Pedaliium murex* L., and *Momordica doica*, which have been traditionally employed for medicinal and food purposes. The plants were grown on complex distillery waste containing a mixture of organometallic compounds. Results revealed bioaccumulation of Mn, Cd, Fe, Cr, Cu, As, Se, Mo, and Co in their roots, shoots, and leaves in levels higher than the surrounding sludge. *A. aspera* was noted as root accumulator for Mn (16.95 mg kg^{-1}), Zn (30.12 mg kg^{-1}), Fe ($240.40 \text{ mg kg}^{-1}$), Co (3.19 mg kg^{-1}), while Se (4.07 mg kg^{-1}), Mo (4.36 mg kg^{-1}), was accumulated selectively in the shoot of the plant. Similarly, *S. bispinosa*, *P. murex*, and *M. doica* were found as root accumulators for Mn, Fe, and Ni. *A. viridis* accumulated Cd, Zn, and Cu in the shoot and leaves of the plant. The high bioconcentration factors (BCF) and translocation factors (TF) observed in these native plants (>1) suggested their tendency to hyperaccumulate heavy metals. The findings highlighted that these plants as a potential metal accumulator may pose health hazards and deteriorate the medicinal property if grown on such wastes.

Keywords: Medicinal plants Heavy metals Androgenic waste Phytoremediation Detoxification

1. Introduction

Heavy metal pollution in soil, water, and food material is a major threat to human health. Industrial wastes and geo-genic activities are the major sources of heavy metals in the environment (Annan et al., 2013; Shammi et al., 2016; Sharma et al., 2021b). In India, several industries like distilleries, tanneries, pulp paper industries, electroplating industries, steel, and iron industries discharge a mixture of heavy metals along with various complex organic wastes into the environment. Due to the complexity in the matrix and non-degradability of the heavy metals, such discharge poses significant challenges for its remediation (Gupta and Sinha, 2007; Chandra et al., 2017; Kumar et al., 2013; Sushil and Batra, 2006). Heavy metals tend to accumulate in the soil, water, and plants in the environment, which could contaminate the food chain subsequently ((Chandra et al., 2009; Sharma et al., 2020b; Sharma and Rath, 2020; Singh et al., 2012). For example, Indian mustard grown in distillery effluent irrigated soil showed significant accumulation of heavy metals (Cd, Cu, Fe, Mn, Ni, and Zn) in their edible parts (i.e. seeds and leaves) and could pose a health risk to humans (Bharagava et al., 2008). It is noteworthy that at $<10\%$ (v/v), the effluent showed an inducible effect on the growth of the mustard plant; while at $>10\%$ (v/v), the effluent showed an inhibitory effect on the various physiological parameters of the plants.

Various physicochemical methods i.e. filtration, flocculation, reverse osmosis, and chemical precipitation have been used for remediation of heavy metals from the terrestrial and aquatic environment, but they are energy-intensive and not cost-effective (Blöcher et al., 2003; Dialynas and Diamadopoulos, 2009; Bratskaya et al., 2009). Phytoremediation has emerged as a promising green technology for the decontamination of metals in polluted sites (Ghassemzadeh et al., 2008; Singh et al., 2017; Sharma et al., 2020a). Recent studies have highlighted the phytoextraction potential of some native plants such as weeds and grasses from metal-contaminated sources and organometallic industrial sludge disposed sites (Gupta and Sinha, 2007; Chandra et al., 2018; Franchi et al., 2017; Sharma et al., 2021a). Among the potential metal accumulators, several are known as food and medicinal plants (Shammi et al., 2016; Singh and Prasad, 2014). According to the World Health Organization (WHO), more than 70% of the modern world's population rely on medicines of herbal origin for their health care (Jaison and Muthukumar, 2017; Annan et al., 2013). The herbal preparation is presumed to be safe from all the contaminants, but studies have shown that they may be contaminated with various heavy metals like cadmium, chromium, lead, mercury, zinc, copper, nickel and manganese, etc. Several metals are intentionally added to the herbal preparation in the form of traditional medicine i.e. Ayurveda, and in many instances, they are artifacts of the manufacturing process. Various leafy herbal plants are known for their applications in medicine and have the inherent ability to bioaccumulate heavy metals, up to several folds higher than the surrounding soil. However, the capacity of plants to accumulate heavy metal may vary within and among the plant species (Jaison and Muthukumar, 2017; Sharma et al., 2020c). The heavy metal contamination in food and medicine could become health hazards and may also change the chemical properties of medicine. Ignorance of heavy metal accumulation in the raw material used in herbal preparation can lead to serious and long-term side effects (WHO, 1978).

Sugarcane molasses-based distilleries are sources of complex environmental pollutants due to the presence of various heavy metal-containing organic pollutants. In India, there are more than 317 sugarcane molasses-based distilleries in operation that generate on average 1500 tonnes of sludge per day during the anaerobic digestion of spent wash (Kansal et al., 1998). This reflects the magnitude of environmental pollution caused by solid waste generated from the distillery sector all over India. These sludges contained high iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), cadmium (Cd), manganese (Mn), nickel (Ni), and lead (Pb) content, all present in concentrations above the prescribed limit in the environment recommended by US Environmental Protection Agency (USEPA). Recent studies highlighted that distilleries sludge contains several persistent organic compounds e.g. dodecanoic acid, octadecanoic acid, n-pentadecanoic acid, hexadecanoic acid, β -sitosterol,

stigmasterol, β -sitosterol trimethyl ether, heptacosane, dotricontane, lanosta-8, 24-dien-3-one, 1-methylene-3-methyl butanol, and 1-phenyl-1-propanol as androgenic and mutagenic compounds. These compounds are listed by the USEPA as endocrine-disrupting chemicals (EDCs) (USEPA, 2012). Due to the complexation of metals with organic compounds, these organometallic compounds restricted the bioavailability of metals to plants. Hence, the generated sludge remains alkaline with a high pollution load of carcinogenic and mutagenic compounds that further impede biodegradation and subsequently damaged the vegetation at the dumping site. Many common native medicinal plants are found to grow well on disposed sludge of distillery waste, however, the accumulation of heavy metals by these plants in the environment from the multi-metal contaminated site is not well studied (Jaison and Muthukumar, 2017; Shammi et al., 2016). To understand the impact of complex industrial wastes in plants and explore their potential for phytoremediation, there is a need to identify the mutagenic and carcinogenic constituents of the distillery sludge and the metal-accumulation patterns in these native plants. Therefore, the main aim of this study is to assess the different metal accumulation patterns by these native plants which are well known for their medicinal use. Recognizing the distribution pattern of heavy metals in various parts or plant tissue will help to promote the correct usage of the plant materials and prevent accidental intake of heavy metals. This study seeks to determine heavy metal accumulation by different leafy medicinal plants from the sugarcane molasses-based distillery waste.

2. Material and methods

2.1. Sample collection and site description

The collection of plants and distillery sludge were carried out in month April to October (2019) from M/s Unnao distillery and breweries, situated in Uttar Pradesh, Unnao, India (26°32'0" N, 80°30'0" E). The distillery unit produces 9000 kilo litre alcohol and generates approx. 800 tonnes of sludge annually (AIDA, 2004). Freshly disposed dried distillery sludges cakes (approx. 5.0 kg) were collected in clean pre-sterilized polythene bags from the sludge dumping site of the distillery plant located inside the premises of the industry. It was treated as the control. Five healthy plants of each species growing on the sludge were collected from the site intermittently with a gap of one month to investigate changes in their properties. The abundant luxuriant growth of six plant species was identified as *Achyranthus aspera* L., *Amaranthus viridis*, *Basella alba* L., *Sesbania bispinosa*, *Pedaliium murex* L. and *Momordica doica* with a standard method according to Duthie flora of Indo-Gangetic plain (Duthie et al., 1903).

2.2. Physico-chemical analysis of sludge before and after plant growth

The physico-chemical parameters e.g. pH, electrical conductivity (EC), chloride (Cl^-), sodium (Na^+), and nitrate (NO_3^-) of the distillery sludge was analyzed and estimated according to the method described by Chandra et al. (2018). The dried sludge cake was treated as a control while fresh sludge samples from the polluted site were used for metal accumulation study. The phenol contents in sludge were determined as per standard methods described by the American Public Health Association (APHA, 2012). The pH and EC values (sludge: water = 1:2.5 w/v) of sludge samples were measured using an Orion pH 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 was measured using atomic absorption spectrophotometry (AAS) (ZEEnit 700, Analytic Jena, Germany) after acid digestion (APHA, 2012). While the BOD, COD was measured in prepared leachate from distillery sludge as described previously (Sharma et al., 2020c).

2.3. Scanning electron microscopy (SEM) and Fourier transform-infrared spectrophotometry (FTIR) analysis of sludge

One gram of distillery sludge was dried in a hot air oven (Evolution-201, Thermo Scientific, USA) at 50 °C overnight till constant weight. The sample was crushed into powder form in a porcelain mortar (Evolution-201, Thermo Scientific, USA) as previously described (Yadav and Chandra, 2018). Further, the sludge sample was treated in 2.5% glutaraldehyde for 3–4 h and postfixed with 1% Osmium tetroxide for 2 h before sputter coated in a gold film and examined under SEM and EDAX (SEM, QUANTA FEG 450, FEI, and the Netherlands) (Yadav and Chandra, 2018). The sample was further analyzed using Fourier transform–infrared spectrophotometry (FTIR) using a spectrophotometer (Nexus–890, Thermo Electron Co., Yokohama, Japan). For elemental analysis of the sludge sample, an area was selected, and the elements in the sediment were examined by a high-resolution SEM equipped with an EDX system (SEM, QUANTA FEG 450, FEI, and the Netherlands). Fourier transform–infrared spectrophotometry (FTIR) analysis of the sludge sample was also performed using a spectrophotometer (Nexus-890, Thermo Electron Co., Yokohama, Japan). The sample was dispersed in spectral-grade KBr (Merck, Darmstadt, Germany) and made into pellets by applying 5–6 tons cm^{-2} of pressure for 10 min using hydraulic pressure (Specac, United Kingdom) instrument. The spectrum was generated in the range of 400 to 4000 cm^{-2} with a resolution of 4 cm^{-1} for all samples (fresh sludge, and a plant grown sample).

2.4. UV–Vis spectral and high-performance liquid chromatography (HPLC) analysis of leachate

The collected sludge samples were pooled, mixed, and then air-dried before ground with a pestle mortar to crush the entire available particle; the crushed samples were sieved through a 63 μm pore

size sieve to get a homogeneous powder. Further, the solvent (ethyl acetate) extraction was carried out to obtain 10% leachate from the sludge as described earlier (Chandra et al., 2008). Briefly, 100 g of sludge was added to 1000 ml of ethyl acetate (w/v) and the mixture was shaken continuously for 3–4 hr at room temperature (25 ± 2 °C), and the suspension was filtered through a 0.22 μ m syringe filter. Furthermore, organic pollutants in the sludge sample were measured using a UV–Vis spectrophotometer (Thermo Fisher Scientific Shanghai spectrophotometer Evolution 2001, China) at a wavelength between 200–700 nm at ambient room temperature (25 °C) an HPLC equipped with a 2487 UV–Vis detector and using Millennium software (Waters 515). The samples (20 μ l) were injected at a rate of 1 ml min⁻¹ using an acetonitrile: water ratio of 70:30. To analyze the compounds at 320 nm by HPLC, a reverse-phase C-18 column (250 $\times$ 4.6 mm, particle size 5 μ m) at 27 °C was used (Chandra et al., 2018).

2.5. Extraction and characterization of residual organic pollutants through GC–MS analysis

2.5.1. Solid–liquid extraction

Various organic compounds from the distillery sludge were extracted using ethyl acetate as per the previously mentioned method (Chandra and Kumar, 2017). The extraction was repeated three times. The organic solvent phase was dried over anhydrous Na₂SO₄, and the solvent was evaporated to dryness using a stream of nitrogen gas at room temperature. Dry organic filters were makeup in 1.5 ml ethyl acetate and filtered through 0.22 μ m syringe filters (Millipore Ltd, Bedford, Massachusetts, USA) and used for GC–MS analysis.

2.5.2. Characterization of organic pollutants

The extracted samples were derivatized with trimethylsilyl (TMS) as described earlier (Chandra and Kumar, 2017). An aliquot (2.0 μ l) of the derivatized sample was injected in a GC–MS instrument (Trace GC Ultra Gas Chromatograph; Thermo Scientific, FL, USA) equipped with a TriPlus auto-sampler coupled to TSQ Quantum XLS triple quadrupole mass spectrometer (Thermo Scientific, FL, USA). Separation of organic compounds occurred in a DB-5MS capillary column. The temperature of the GC oven started from 65 °C (hold for 2.0 min), increased to 230 °C at a rate of 6 °C min⁻¹, and finally reached 290 °C (hold for 20 min) at the rate of 10 °C min⁻¹. Helium was used as carrier gas at a flow rate of 1.1 ml min⁻¹. The mass spectrum (MS) was operated in the positive electron ionization (+EI) mode at 70 eV. The detected organic compounds extracted from fresh and plant grown samples were identified by matching with the MS library NIST version 1.0.0.12 available with the instrument.

2.6. Digestion of plants for metals estimation

To estimate the metal content accumulated in the potential plant tissue of native plants, the uprooted plants were washed thoroughly with deionized water to remove all the sludge particles from the roots, followed by rinsing with a 10 mM solution of calcium chloride. Subsequently, the plant's roots, shoots, and leaves were separated and chopped into small pieces, and the resulting biomass was oven-dried at 70 °C for 5 days till constant weight. The dried plant parts were washed in a muffle furnace at 460 °C for 6 h. The weighted ash from these samples was digested in 2% nitric acid (HNO₃) and filtered through a 0.45 µm glass fiber filter (AOAC, 2002). One gram (1.0 g) of dried and sieved sediments was digested with 10 ml of HNO₃. If brown fumes appeared, additional 5 ml of HNO₃ was added and digestion continued till no generation of brown fumes. The concentrations of Cr, Zn, Mn, Ni, Cu, Fe, Cd, and Pb were determined by AAS (Chandra et al., 2017; Sharma et al., 2020a).

2.7. Metals accumulation efficiency

To evaluate the metal accumulation efficiency in plants, the bioaccumulation coefficient factor (BCF) and translocation factor (TF) were calculated. BCF is defined as the ratio of metal concentration in the root to the soil, and TF is the ratio of metal concentration in the shoot to the root. BCF and TF were calculated according to the formulae below which have also been reported earlier by Yoon et al. (2006).

$$BCF = \frac{C_{root}}{C_{sludge}} \quad (1)$$

$$TF = \frac{C_{shoot}}{C_{root}} \quad (2)$$

Where, C_{root} = concentration of metal in plant root (mg kg⁻¹), C_{sludge} concentration of metal in distillery sludge (mg kg⁻¹), and C_{shoot} concentration of metal in mg kg⁻¹ as per the dry weight of plant shoot. Both BCF and TF have to be considered for evaluating whether a plant is a metal hyperaccumulator.

2.8. Histological observations of root tissues by transmission electron microscopy

Root segments (2.0 mm in length) of selected plants were immersed in H₂S saturated water as pre-treatment for 30 min at room temperature to precipitate trace elements. The root sample was washed with 0.1M SCB (sodium cacodylate buffer, pH 7.2) and fixed in 2.5% glutaraldehyde (v/v) prepared in sodium cacodylate (Ladd Research Industries, Williston, USA) buffer (pH 7.2) for 2 h at 4 °C. The root tissue was washed three times with 0.1M SCB with a 10 min interval between each washing and postfixed in 1% OsO₄ overnight. The fixed tissue was washed with SCB, dehydrated in

graded acetone series, and embedded in the Araldite-DDSA mixture (Ladd Research Industries, Williston, USA). After backing at 60 °C, blocks were cut (60–80 nm thick) by an ultra-microtome (Cryo Leica EM UC7, Leica Microsystem, India), and the sections were stained by uranyl acetate and lead citrate. Analysis of the section was done under transmission electron microscopy (TEM) (FEI Tecnai™ G2 Spirit Twin, Hillsboro, USA) at an accelerating voltage of 80 kV (Chandra et al., 2018; Sharma et al., 2020b).

2.9. Statistical data analysis

In-situ phytoremediation process and metal accumulation compared with the original distillery sludge (pre-treatment) and Student's *t*-test ($p < 0.001$) was carried out. To confirm the variability of data obtained and the validity of results, the mean concentration of heavy metals in the root, shoot, and leaves were subjected for the ANOVA analyses (Ott, 1984). All statistical analyses were carried out using the SPSS statistical software (version 17.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Physico–chemical characterization of fresh and after plant growth sample of sugarcane molasses sludge

All the physico–chemical parameters in leachate and sludge of the distillery waste were above the permissible limits as shown in Table 1. The BOD/COD ratio indicated low degradability due to the presence of recalcitrant compounds (Kumar et al., 2010). Different metals (i.e. Mn, Cr, Zn, Cu, Fe, Pb, Cd, and Ni) were also found in concentrations higher than the USEPA guideline (2012). The high content of heavy metals in distillery sludge might be due to the corrosive effect of sugarcane juice in sugar manufacturing industries and the boiling of the juice during molasses separation, fermentation, and distillation process of sugar cane molasses in the distilleries. In the subsequent step, the metals might be leached into the spent wash from the drainage pipe due to high acidic conditions. The leaching of heavy metal in the acidic medium in the industrial process is well documented in a previous study (Noor and Al-Moubaraki, 2008).

While after plant growth, various pollution parameters, phenol, and potassium, phosphate, nitrogen contents, and the metal contents were significantly reduced from fresh sludge (Table 1). These data provided a strong indication of the phytoextraction capability of heavy metals and the phytoremediation potential of organic pollutants of the native plants. The repeated application of metal-containing industrial effluent in irrigation also showed the accumulation of toxic metals in the edible parts of crop plants (Chandra et al., 2009). The high content of heavy metal in distillery sludge and their adverse effect on seed germination and growth parameter of green gram *Phaseolus mungo*

L. were reported in a study by Chandra et al. (2008). However, in the current study, the native medicinal plants did not appear to be affected negatively by the elevated metal contents, further suggesting their tolerance to heavy metals. The high affinities of metallic ions with organic pollutants are known to restrict the bioavailability of metals to plants (Migo et al., 1997). The ability of the plants to accumulate heavy metals might be due to increased solubility and availability of metal resulting from the plant microbe's interaction (Rajkumar et al., 2012).

The increase of electric conductivity after plant growth on the distillery sludge indicated the function of ions (cations/anions) present in sludge (Bose and Bhattacharyya, 2008; Sinha et al., 2007). The reduction of total nitrogen suggested nitrogen was taken by the plant as a nutrient for growth; it could also be attributed to the conversion of nitrate to ammonium with a mixed reaction of microbes and plants. This finding collaborated with previous observations of plant growth on the sludge of distilleries (Chandra and Kumar, 2017). Similarly, the BOD, COD values in the sludge leachate also reduced after plant growth. This result clearly showed phytoremediation along with the phytoextraction of heavy metals from sludge.

3.2. Morphological view, element components, and functional groups analysis of sludge

The SEM image of sludge showed fine crystals in needle shape. Some scattered elongated crystals were noted as well as the glomerated needle-shaped fine crystals as shown in Fig. 1A. The needle-like crystal properties have been reported as organic polymers and metallic crystals (Liu et al., 2013). The elemental analysis in EDX showed the presence of carbon, oxygen, sodium, potassium, calcium, and surface in different percentages as shown in Fig. 1B. The IR spectra of the sludge pre-plant growth showed medium and strong bonds of the compounds with the different stretching group at 3426.40 cm^{-1} (N-H), 2967.1 (C-H), 2470.5 ($\text{O}=\text{C}=\text{O}$), 1644.3 (C=N), 1559.7 (N-O), 1446.4 (C-H), 1411.6 (S=O), 1196.1 (C-O), 1048.3 (CO-O-CO), 866.7 (C-H)(Fig.S1A& Table.S1B). While the post-plant growth FTIR data showed maximum strong bond stretching frequency of compounds 3415.5 (O-H), 2967.1 (C-H), 1564.6(N-O), 1415.5 (S=O), 1211.0 (C-O), 1049.3(CO-O-CO), 1021.1($\text{C}\equiv\text{C}$), 654.4(C-Br). This suggested that the presence of various compound classes, such as carbon dioxide, sulfonyl chloride, ester, and anhydride. After plant growth, most of the medium bonds of detected compounds were broken; in contrast, compounds with strong bonds remained intact (Table S1.A& Fig.S1.B).

3.3. UV-Vis spectral and HPLC analysis of leachate

Analysis of the UV-Vis absorption spectra at wavelength 250–700 nm showed the presence of variable peaks in the UV-range due to the dissolved organic compounds in the sugarcane molasses-

based distilleries sludge before phytoremediation; these peaks were diminished after plant growth as shown in Fig. 1C. The presence of soluble organic matter in the UV–Vis range was noted. The sludge sample before plant growth showed various mixed peaks which indicated a mixture of pollutants present in the leachate with absorption maxima at 320 nm. While after the plant growth, many of the absorption peaks disappeared signifying the degradation of various organic pollutants. In this analysis, the integrated UV absorption reflected the overall volume of aromatic or unsaturated compounds and their double bond absorption i.e. C=C, C=O, and N=N. The spectra (Fig. 1C) were produced by calculating the absorption of monochromatic radiation through a spectrum of wavelengths in the pre- and post-phytoextraction solution; it was one of the most common techniques for monitoring pollutants from sludge (Chandra et al., 2018). The comparative chromatograms of HPLC before and after phytoextraction were shown in Fig. 1D. The reduction in the major pollutant suggested the degradation of organic pollutants in the sludge and phytoextraction process by plants, which removes complex organic compounds and most of the heavy metals that are toxic to the biota of the environment even at low concentrations. A similar pattern on the phytoextraction process from distillery sludge has been shown by other plants in previous studies (Chandra et al., 2018).

3.4. Identification of organic pollutants

The GC–MS analysis in ethyl acetate extract of distillery sludge of control sample (bulk sludge) and rhizospheric soil after plant growth on sludge was analyzed to investigate the alteration of organic compounds. 3.4.1. Identification of organic pollutants (bulk sludge) The major peaks of an extracted sample (bulk sample) were observed at RT 6.77, 13.17, 13.56, 19.12, 20.50, 23.99, 25.00, 27.28, 30.16, 31.18, 35.04, 48.22 as shown in Fig. S2 A. These compounds were characterized as acetamide, 2,2,2-trifluoro-N-methyl, benzene acetic acid, TMS ester, butanedioic acid, bis(TMS)ester, hexadecane, dodecanoic acid, TMS ester, anthracene, dotricontane, ethanol, 2-(octadecyloxy), octadecanoic acid, TMS ester, hahnefett, nonacosane as described by the NIST library available with the instrument. Moreover, other minor peaks were also noted at RT values of 8.05, 17.20, 35.37, 36.69, 39.66, 40.52, 42.64, 49.02, and 51.12 as shown in Fig.S2. These compounds were characterized as butane, 2,3-bis(TMS oxy), α -ketoglutamic acid, bis(TMS)ester, nonacosane, stigmata-5,22-dien-3-ol (3 β , 22E), stigmasterol, lanosta-8,24-dien-one, β -sitosterol, silane, [(3 β)-cholest-5-en-3-yl]oxy]TM, methylene bis (2,4,6-triisopropyl phenyl phosphine). Most of the identified compounds were classified either under the category of plant fatty acids or chemical reaction by-products of the distillation process (Chandra et al., 2018). The toxicities of detected pollutants were reported in Table 2. The source of complex phenolic compounds in sludge might be from the extract of sugarcane molasses after fermentation. Different forms of detected aromatic compounds could be the result of carbonyl and

amine group reaction at elevated temperature in the sugar industry, which remains as complex in cane molasses known as melanoidin (Chandra et al., 2018). The presence of several fatty acids in the sludge might contribute to the reduced rate of metal accumulation processes and biotransformation processes of complex organometallic compounds in plants. When these organic pollutants reach the aquatic ecosystem, they can cause toxic effects on flora and fauna of water bodies. Recently, some of the detected plant organic residues (phytosterols) such as stigmasterol-5, 22-dien-3-ol (3 β , 22E), stigmasterol, lanosta-8, 24-dien-one, β -sitosterol, and silane have been demonstrated to be toxic to the aquatic ecosystem. Other organic acids like octadecanoic acid, dodecanoic acid, and butanedioic acid are also listed as endocrine disruptors. The presence of EDCs compounds in distillery sludge has given strong evidence for the complex nature of sludge with various toxic compounds. These compounds are either generated at the time of fermentation or during the anaerobic treatment of distillery sludge at the disposed site (Chandra et al., 2018). Many of these detected compounds caused adverse effects in environmental flora and fauna of soil as well as the aquatic ecosystem.

Table 1. Physico-chemical characteristics of discharged distillery fresh sludge before and after plant growth collected from M/s Unnao Distillery Pvt. Ltd. *Unnao*, Uttar Pradesh, India.

Parameters	Sludge before plant growth	Sludge after plant growth	Reduction %	Permissible limit (USEPA, 2012)	CPCB (2017)
Color appearance	Blackish Brown	Brown	–	–	–
Odor	like molasses	Like molasses	–	–	–
pH	8.67 ± 0.16	7.95 ± 0.22 ^a	94.5%	8.00 ± 0.01	7.54 ± 0.01
Biological oxygen demand (BOD) (mg/L ⁻¹)	4166.82 ± 88.22	2500.11 ± 86.60 ^a	60.0%	40.00	47.00 ± 0.00
Chemical oxygen demand (COD) (mg/L ⁻¹)	12527.18 ± 182.22	6850.84 ± 128.11 ^a	54.68%	121.00	79.00 ± 0.01
Electrical conductivity (EC)	1916.66 ± 60.09	1003.33 ± 31.79 ^a	50%	1000	950
Total Dissolve solid (TDS) (mg/L ⁻¹)	10720.78 ± 260.44	5521.99 ± 151.29 ^a	51.3%	50–70	70 ± 0.00
VS (mg/L ⁻¹)	1214.84 ± 36.01	515.84 ± 14.60 ^b	41.16%		20 ± 0.01
Chloride (mg/L ⁻¹)	2935.33 ± 70.95	1193.33 ± 52.06 ^b	40.45%	750.00	11.82 ± 0.01
Total Nitrogen (TN) (mg/L ⁻¹)	228.51 ± 8.78	141.81 ± 5.87 ^a	57.7%		9.90 ± 0.00
Phenol (mg/L ⁻¹)	8015 ± 73.99	2702.33 ± 35.89 ^c	33.33%	0.50	–
Sulphate(mg/L ⁻¹)	15,616.95 ± 606.47	8557.15 ± 83.36 ^b	55.31%	750.00	
Phosphate (mg/L ⁻¹)	48.04 ± 1.52	21.90 ± 0.92 ^b	51.97%	–	3.40 ± 0.01
Heavy metals					
Mn (mg L ⁻¹)	8.75 ± 0.52	3.58 ± 0.22 ^b	40.98%	0.20	0.15
Cr (mg L ⁻¹)	3.73 ± 0.38	1.06 ± 0.12 ^c	26.0%	0.05	0.01
Zn (mg L ⁻¹)	18.84 ± 0.89	8.23 ± 0.67	35.10%	2.00	1.28
Cu (mg L ⁻¹)	3.91 ± 0.21	0.50 ± 0.08 ^{NS}	12.56%	0.50	0.19
Fe (mg L ⁻¹)	423.88 ± 7.33	186.44 ± 8.89	41.46%	2.00	1.45
Pb (mg L ⁻¹)	5.08 ± 0.27	1.86 ± 0.25 ^c	32.81%	0.05	0.02
Cd (mg L ⁻¹)	BDL	BDL	BDL	BDL	BDL
Ni (mg L ⁻¹)	8.78 ± 0.64	3.91 ± 0.58 ^a	40.14%	0.10	0.04
Na (mg L ⁻¹)	488.28 ± 6.68	63.25 ± 3.7 ^{NS}	20.49%	0.04	0.01
K (mg L ⁻¹)	245.15 ± 10.30	136.99 ± 1.99	60.86%	0.09	0.02

All the values are Mean ± SE. (n = 3); Unit of all parameters is in (mg L⁻¹) except pH, color (Co-Pt. Unit) and EC (μmhoscm⁻¹); Students *t* test (two tailed as compared to pre-treated sludge); BDL: Below detection Limit.

^a Highly significant at $p < 0.001$; ^b Significant at $p < 0.01$; ^c Less significant at $p < 0.05$; ^{NS} Non-significant at $p > 0.0$

Fig. 1. A. Scanning Electron Microscopy analysis of distillery waste sludge sample; 1B: EDAX of distillery waste sludge sample; 1C: UV–Vis spectral analysis of before and after phytoextraction at various time intervals; 1D: HPLC analysis of before and after phytoextraction.

3.4.2. Identification of organic pollutants from distillery sludge after plant growth

Detection of organic compounds from phytoextracted distillery sludge was analyzed in detail (Table 2). The number of organic compounds and peaks were reduced in the sludge after plant growth as compared to the control sludge sample. The major peaks of the phytoextracted sample were noted at RT 8.66, 12.82, 13.78, 16.01, 20.99, 21.91, 22.18, 22.81, and 22.90 as shown in Fig. S2B. These compounds were characterized as 1,3-propanediol, TMS ether, 5-methyl-2-(1-methyl ethyl) cyclohexanol, butanedioic acid, bis(TMS) ester, 2,3-butanediol, bis-O-(TMS), dodecanoic acid, TMS ester, butanedioic acid, bis(TMS) ester, cyclooctene, 1,2-bis(TMS), tricarboxylic Acid TMS. Similarly, other minor peaks were also detected at RT values of 9.88, 10.27, 11.54, 13.95, 14.98, 17.53, 17.72, 19.10, 20.08, 24.88, 25.96, 26.49 and 27.84. These compounds were characterized as propanoic acid, 3-[(TMS) oxy, 1, 3 propanediol, TMS ether, pentanoic acid, malic acid (O-(TMS)-bis (TMS ester), tert-butyl hydroquinone, bis (TMS), vanillin propionic acid, bis (TMS), tricarballic acid 3 TMS, benzene acetic acid according to the NIST library. The result showed that original organic compounds present in distillery sludge before plant growth were either degraded or biotransformed into new compounds

as shown in Table 2. This confirmed the medicinal plants harvested from the contaminated were able to bioremediate the distillery sludge. The bioremediation along with the phytoextraction of heavy metals by the other native weeds has shown similar observation and it was found as potential metal accumulation from complex organometallic wastes (Chandra et al., 2018).

3.5. Accumulation of total heavy metals in selected plants

To assess the potential of using native plants for phytoremediation of distillery sludge, the effect of metal accumulation by six native medicinal plants were further investigated. Six native medicinal plants (i.e. *Achyranthus aspera* L., *Amaranthus viridis*, *Basella alba* L., *Sesbania bispinosa*, *Pedaliium murex* L., and *Momordica doica*) growing at the discharge site of distillery sludge were analyzed for their accumulation of twelve heavy metals (Mn, Pb, Cd, Zn, Cr, Fe, Cu, Ni, As, Se, Mo, and Co). The variable pattern of metal accumulation to the various parts of the plant species indicated different capacities of metal uptake as shown in Table 3 and Fig. S3 and S4. A previous study showed that the metal contents along with organic compounds in distillery sludge inhibited the development of the roots of various crops and stunted the growth of their shoot (Mazumdar and Das, 2015; Gupta and Sinha, 2007). However, the luxuriant growth of plants along with a well-developed root system indicated the inherent potential of the native plants due to their genetic property. As the distillery sludge had a complex matrix, the presence of various organic compounds could reduce the bioavailability of the multi-metals to the native plants.

The overall accumulation pattern of different metals in various plants was noted as below:

The accumulation of various heavy metals by *A. aspera* L. has been reported from a separate study (Saraf and Samant, 2013). Similarly, another study showed Fe and other metals accumulation in plants from the lignocellulosic waste containing multi-metal complex (Chandra et al., 2017). Excess Fe was observed in this study in the root of the plants. From a toxicological point of view, although Fe is an essential element, excess level could cause phytotoxicity, the ability of *A. viridis* to accumulate up to 830 mg kg⁻¹ Fe indicated the presence of metal detoxifying mechanisms and the potential of the plant as a phytoremediation candidate. A similar pattern for the accumulation of Mn and Fe has been reported in the root of *A. aspera* L. in a previous study (Saraf and Samant, 2013). The study highlighted the accumulation of high content of Fe, Cu, Co, and Au from metal contaminated soil. *S. Bispinosa* was also reported to be able to accumulate a high concentration of lead (Sahi et al., 2002). Overall, the six tested plant species showed a high concentration of metal accumulation as compared to the normal uptake of metal, this indicated that these plants were capable to grow at the contaminated site of distillery sludge and could play a vital role in phyto-accumulation of heavy metals.

The accumulation of heavy metal depends upon the plant species, distribution, and variability of the microbial community, the chemical property of organic pollutants, and the pH of a substrate (Laghlimi et al., 2015; Kumar et al., 2013; Gupta and Sinha, 2006; Sharma et al., 2020b). The disposed distillery sludge after anaerobic digestion became slightly alkaline ($\text{pH } 8.67 \pm 0.16$) which could restrict metal availability to plant. But the metal accumulation by growing plant on sludge showed the potentiality of plant and there was a gradual decrease of pH in the rhizospheric soil. This might be due to the plant microbes' interaction in the rhizospheric zone, there is an abundant release of acids from root hairs like gluconate, 2-ketogluconate, oxalate, citrate, acetate, malate, and succinate (Ma et al., 2011). Our result indicated that the distillery sludge as waste comprised various organic compounds and metals that formed organo-metallic complexes. Most of the compounds in sludge were anionic which has a strong cationic metal-binding tendency (Migo et al., 1997). Improved bioavailabilities of metals to plant by growing rhizospheric bacterial communities have also been reported previously (Sessitsch et al., 2013; Rajkumar et al., 2012).

3.6. Bioconcentration factor and translocation factor

The ability of heavy metals accumulation for plants can be evaluated through the BCF and TF (Yoon et al., 2006). The BCF of the plants grown in distillery sludge indicated that all the plants had a high ability for phytoextraction of metals in their root. In general, the bioavailability of heavy metals in sludge is very poor but all the tested plants shown very high metal accumulation in their different parts which indicated the hyperaccumulation tendency of these plants (Table 4).

Metal concentration-dependent accumulation in plant parts has been reported by several studies in aquatic and terrestrial soil (Yoon et al., 2006; Bharagava et al., 2008). In this study, the metal accumulation pattern was in order roots > shoots > leaves. This indicated the concentration-dependent mobility of metals in plant tissues (Gupta and Sinha, 2006). *Achyranthus aspera* L. exhibited high potential for the accumulation Cu ($76.22 \pm 1.3 \text{ mg kg}^{-1}$), Mn ($9.80 \pm 0.6 \text{ mg kg}^{-1}$), Cr ($25.9 \pm 1.3 \text{ mg kg}^{-1}$), Pb ($7.99 \pm 0.7 \text{ mg kg}^{-1}$), and Zn ($67.33 \pm 0.6 \text{ mg kg}^{-1}$) with the highest BCF in five out of eleven metals (Table 3). *Amaranthus viridis* displayed maximum BCF for Cu followed by Cr and Pb. Different heavy metals accumulation potential of *A. aspera* L. had been reported from the soil with different characteristic properties (Vijaya Kumar et al., 2009). These studies showed that *A. aspera* L. has a propensity to the accumulation of Fe, Cu, Mn, Na, in higher amount. To our knowledge, this is the first report on the metal accumulation pattern of *A. aspera* L. from organometallic sludge. *Basella alba* L. also showed a high accumulation of Fe, Cu, and As in root, but higher arsenic concentration was noted in the shoot (50.01 mg kg^{-1}). The higher accumulation tendency for Na, Cu, and Mn of *B. alba* L. has been reported in other studies (Shammi et al., 2016). However, the metal accumulation pattern from

industrial sludge and its impact on medicinal quality is still not clear. The higher accumulation of Mn ($40.76 \pm 0.8 \text{ mg kg}^{-1}$), Zn ($77.06 \pm 1.6 \text{ mg kg}^{-1}$), Fe ($879.0 \pm 1.5 \text{ mg kg}^{-1}$), As ($31.81 \pm 1.3 \text{ mg kg}^{-1}$) and Mo ($20.35 \pm 0.3 \text{ mg kg}^{-1}$) in root and shoot of *S. bispinosa*. *Pedaliium murex* L. showed high accumulation of all the tested metals in their root, shoot, and leaves from sludge this indicated most potential plant for phytoextraction of metal from the organometallic complex shown in Table 3. This might be due to root nodule forming bacteria of the plant which may be favorable for metal bioavailability in sludge to plant.

High BCF and metal accumulation have been reported in the previous study from the metal-contaminated site (Saraf and Samant, 2013). *Basella alba* L. showed a high BCF for Mn & Mo followed by Pb and Cu which indicated the potentiality of the plant for metal accumulation from any contaminated site (Table 4) (Shammi et al., 2016). The deep and strong growth of roots also supported the potentiality of the plant. A similar pattern was observed in *S. bispinosa*, *P. murex* L., and *M. doica*. The maximum translocation factor of Cu and Co was found in *Amaranthus* and *Sesbania*, respectively. The minimum TF was noted in *Achyranthus* and *Momordica* for a nickel. The $\text{TF} > 1$ indicated the high physiological and transpiration rate of the plant. Concomitantly, these plants have shown their adaptability to accumulate a higher concentration of metals from distillery sludge. The accumulation of metals by plants from sludge to root depends on the chemical nature of the element, pH, and other co-pollutants of sludge that inhibits the mobility of metals in plants (Gupta and Sinha, 2008).

The high translocation and bioconcentration of metals in different parts of the medicinal plants is a health hazard as metals accumulate in shoots and leaves that are traditionally harvested for medicinal purposes. When textile effluent was reused as irrigation water, *B. alba* L. was found to pose a health risk due to the accumulation of high levels of heavy metals (Shammi et al., 2016).

3.7. Observation of metal accumulation in root tissue of selected plants

The root tissue analysis of collected indigenous hyperaccumulator plants by transmission electron microscope shown metal accumulation in vesicle near the cell wall and cytoplasm (Fig. 2A-F). The metal granules were deposited near the nucleus in *A. aspera* L. and *S. bispinosa*; while big granules were observed in the cytoplasm and near the cell walls in *A. viridis*, *B. alba* L. and *M. doica* (Fig. 2). The continuous deposition of fine metal granules at the cell wall regions was noted in *P. murex* L. This study concluded that the mechanism of plant detoxification of heavy metals was based on vacuole sequestration and cell wall deposition (Fig. 2). *Basella alba* L. and *S. bispinosa* root tissues showed the presence of multi-nucleolus, multi-vacuoles, thickened in the center, and round the lamellae with metal accumulation on the cell wall, cytoplasm, and middle lamella. The development and deposition

of metal granules in cell walls indicated a plant resistance mechanism for metal aggregation and detoxification with a larger volume of cellular tissues (Tong et al., 2004).

The phytoextraction potential of some native plants growing on distillery sludge reported in a previous study also showed a close resemblance to the multi-vacuoles development and deposition of granules, as an adaptive mechanism of the plants in the presence of organic pollutants and metal accumulation. But the pattern of a multi-metal accumulation from industrial waste by medicinal plants has not been reported. The ultra-structural observation of root tissue of *P. murex* L., *M. doica* at low and high magnification showed deposition of metal granules inside the cell walls, cell membranes, cytoplasm, and nucleoplasm (Najeeb et al., 2011). The whole plant root also showed cell wall thickening. Increased development of nucleolus and vacuoles at high heavy metal concentration increased the output of the ribosome and mRNA, which finally increased the development of fresh proteins engaged in the tolerance of heavy metals in plants (Najeeb et al., 2011). In our research, all species of plants were noted with extravagant growth on disposed distillery sludge without any biochemical deformities in their aerial parts. These plants showed good adaptability to survive and develop in organo-metallic and EDCs containing distillery sludge and are suitable for in situ phytoremediation. They are good candidates for the monitoring of heavy metals in intricate and dangerous industrial wastes and eco-restoration polluted areas.

Fig. 2. Electron micrographs of transverse section of plants root after phytoextraction of heavy metals (A–F), V: Vacuole; PM: Plasma membrane; P: Peroxisomes; CW: Cell wall; CM: Cell membrane; ML: Middle lamella; N: Nucleus; Arrow ( ) indicated metals deposition; IS: Intercellular space. PL:- Plasmalema.

Table 2 Detection of residual organic pollutants by GC–MS analysis from distillery sludge waste before and after phytoextraction. *RT-retention time (in minutes), + present, – absent, (TMS) trimethylsilyl.

RT:	Name of Compound before extraction	Molecular Formula	Abundance (%)	Nature of compounds	% similarity (NIST Library)	Toxicity
6.77	Acetamide, 2,2,2-trifluoro-N-methyl	C ₆ H ₁₆ O ₂ Si	98	Organic Nature	75.95	slight skin irritant, strong eye irritant,
7.10	2-(2-Hexyloxyethoxy)ethanol	C ₁₃ H ₃₂ O ₄ Si	76	Sulfonic benzoic	90.23	vomiting and diarrhea
8.05	Butane, 2,3-bis(TMSoxy)	C ₁₂ H ₂₈ O ₃ Si	68	Alkane Nature	87.18	gastrointestinal (Digestive), Hepatic (Liver)
11.85	Hexanoic acid, 2-[(TMS)oxy]	C ₁₂ H ₂₂ O ₃ Si ₂	70	Fatty acid	78.01	Hematological (Blood Forming),
13.17	Benzeneacetic acid, TMS ester	C ₁₀ H ₂₆ O ₂ Si	52	Sulfonic benzoic	93.11	Aquatic Toxicology
13.56	Butanedioic acid, bis(TMS)ester	C ₁₃ H ₃₀ O ₅ Si ₃	45	Fatty acid	93.12	Sore throat. Skin & Eye Redness.
15.49	Pentanedioic acid, bis(TMS)ester	C ₁₄ H ₂₄ O ₃ Si ₂	61	Fatty acid	57.01	Skin, Eye, and Respiratory Irritations
16.64	Decanoic acid, TMS ester	C ₉ H ₂₂ O ₃ Si ₂	98	Fatty acid	98.23	Irritation when applied to human skin
17.20	α-Ketoglutaric acid, bis(TMS)ester	C ₁₉ H ₄₀ O ₂ Si	59	Metabolic product	90.02	Neurological brain disorder
19.12	Hexadecane	C ₂₁ H ₄₄ O ₂ Si	73	Alkane Nature	97.13	Dizziness, headache and vomiting
20.50	Dodecanoic acid, TMS ester	C ₁₉ H ₃₆ O ₅ Si	68	Fatty acid	92.05	Ingestion, inhalation & skin absorption
21.40	Quercetin 7,3',4'-Trimethoxy	C ₁₀ H ₂₄ O ₃ Si	94	Saturated FA	74.35	chronic diseases
23.99	Anthracene	C ₁₁ H ₂₈ O ₂ Si	93	Organic Nature	34.54	Melanoma Aspiration hazard
25.00	Dotricontane	C ₁₂ H ₃₆ O ₄ Si ₅	80	Saturated FA	95.14	Sensitization, Skin
27.28	Ethanol, 2-(octadecyloxy)	C ₁₂ H ₃₂ O ₃ Si ₃	81	Organic Nature	71.23	Reproductive toxicity.
30.16	Octadecanoic acid, TMS ester	C ₂₁ H ₄₄ O ₂ Si	31	Fatty acid	65.00	Carcinogenicity
32.01	Tetracosane	C ₂₇ H ₅₈ O ₄ Si	54	Organic Nature	67.98	aquatic environment, long-term hazard
32.40	Eicosanoic acid, TMS ester	C ₃₁ H ₅₆ O ₅ Si	68	Acyclic Nature	97.24	headaches, drowsiness, confusion
35.04	Hahnefett	C ₃₂ H ₅₆ O ₅ Si	42	Alkane Nature	93.11	seizures, and life-threatening complications
35.57	Nonacosane	C ₃₂ H ₅₈ O ₅ Si	55	Alkane Nature	89.74	breakdown of the hemoglobin
(RT)	Name of Compound after extraction	Molecular Formula	Abundance (%)	Nature of compounds	% similarity (NIST Library)	Toxicity
8.66	1,3-Propanediol, TMS ether	C ₆ H ₁₆ O ₂ Si	53	Acyclic	76.34	chronic poisoning, weakness, muscle aches
9.88	Propanoic acid, 3-[(TMS)oxy]	C ₉ H ₂₂ O ₃ Si ₂	97	Fatty acid	96.03	Skin and nail symptoms
10.27	1,3 Propanediol, TMS ether	C ₆ H ₁₆ O ₂ Si	78	Alkane Nature	89.02	hyper pigmentation
11.54	Pentanoic acid	C ₁₂ H ₂₈ O ₃ Si	76	Fatty acid	24.22	inflammation of sensory and motor nerve
13.78	Butanedioic acid, bis(TMS) ester	C ₁₀ H ₂₂ O ₄ Si ₂	98	Fatty acid	75.74	Hemolysis, anemia, hypotension
15.52	Resorcinol, O-bis(TMS)	C ₁₂ H ₂₂ O ₂ Si ₂	76	Saturated FA	98.54	low level of iron in the red blood cells
16.01	2,3-Butandiol, bis-O-(TMS)	C ₁₀ H ₂₆ O ₂ Si	68	Sulfonic benzoic	90.75	low blood pressure
17.53	Malic acid (O-(TMS)-bis(TMS ester)	C ₁₃ H ₃₀ O ₅ Si ₃	79	Saturated FA	98.23	abdominal pain; fever; and diarrhea
21.91	Cyclooctene, 1,2-bis(TMS)	C ₁₄ H ₃₀ O ₂ Si	68	Acyclic Nature	95.64	encephalopathy)
22.18	Tricarballic Acid TMS	C ₁₅ H ₃₂ O ₆ Si ₃	69	Organic Nature	78.35	nerve disease of the extremities
23.54	Benzoic acid,	C ₁₆ H ₃₀ O ₄ Si ₃	79	Acyclic Nature	86.18	loss or deficiency of the fatty coverings
24.88	Tert-butylhydroquinone, bis (TMS)	C ₁₆ H ₃₀ O ₂ Si ₂	58	Sulfonic benzoic	73.65	stomach tumors and damage
26.49	Vanillylpropionic acid, bis (TMS)	C ₁₇ H ₃₂ O ₃ Si ₂	65	Fatty acid	95.12	Skin corrosion/irritation
27.83	Tricarballic acid 3 TMS	C ₁₅ H ₃₂ O ₆ Si ₃	76	Fatty acid	93.16	specific organ toxicity, Respiratory tract irritation
30.61	Benzeneacetic acid	C ₁₅ H ₂₆ O ₄ Si ₂	99	Saturated FA	99.00	Cough sore throat. Skin redness. Eyes pain

*RT-retention time (in minutes), + present, – absent, (TMS) trimethylsilyl

Table 3. Heavy metal accumulation (mg kg⁻¹ DW) in the root, shoots, and leaves of various hyperaccumulator plant species growing contaminated site of distillery waste site. All the values are mean of three replicates (n = 3) ± standard deviation (SD), BDL: below detection limit, R: Root, S: Shoot, L: Leaf.

Plant	Plant part	Mn	Pb	Cd	Zn	Cr	Fe	Cu	Ni	As	Se	Mo	Co
<i>A. aspera</i>	Root	16.95±0.1	2.45±0.4	4.76±0.2	30.12±0.3	3.65±0.2	240.4±0.1	23.25±0.3	3.87±0.5	4.02±0.1	3.67±0.1	2.57±0.2	3.19±0.2
	Shoot	13.35±0.4	Nil	Nil	17.87±0.1	15.54±0.6	345.9±0.5	35.45±0.4	1.83±0.1	1.98±0.5	4.07±0.3	4.36±0.2	2.34±0.3
	Leaves	12.25±0.3	5.54±0.3	3.32±0.3	19.34±0.2	6.76±0.5	538.7±0.1	17.52±0.6	4.59±0.6	3.03±0.1	1.87±0.3	2.87±0.2	2.98±0.2
	Total	42.55±0.8	7.99±0.7	8.08±0.5	67.33±0.6	25.9±1.3	1125±0.7	76.22±1.3	10.29 ± 1.2	9.03±0.7	9.61±0.7	9.80±0.6	8.51±0.7
	Accumulation pattern	R>S>L	L>R>S	L>R>S	R>L>S	S>L>R	R>S>L	S>R>L	L>R>S	S>R>L	S>L>R	S>R>L	R>L>S
<i>A. viridis</i>	Root	56.02±0.2	4.65±0.5	1.87±0.4	23.38±0.7	6.78±0.5	497.6±0.3	9.56± 0.9	5.78±0.7	5.70±0.7	6.21±0.8	5.45±0.5	6.25±0.2
	Shoot	Nil	3.65 ± 0.6	1.54±0.7	35.99±0.6	7.98±0.5	117.5±0.5	16.12± 0.7	3.76±0.6	4.87±0.7	7.28±0.7	4.65±0.4	5.34±0.3
	Leaves	9.98±0.5	Nil	2.43±0.5	34.87±0.4	5.93±0.1	215.3±0.2	20.76± 0.4	5.54±0.5	3.56±0.8	5.44±0.3	5.74±0.3	6.44±0.6
	Total	66.00±0.7	8.30±1.1	5.84±1.6	94.24±1.7	20.69±1.1	830.4± 1.0	46.44±2.0	15.08±1.8	14.8±2.2	18.93±1.8	15.84±1.2	18.03±1.1
	Accumulation pattern	R>L	R>S	L>R>S	L>S>R	S>R>L	R>L>S	L>S>R	R>L>S	R>S>L	R>S>L	R>S>L	R>L>S
<i>B. alba</i>	Root	48.96±0.3	0.98± 0.7	1.23±0.7	42.65±0.3	5.67±0.2	375.4± 0.3	57.61±0.4	5.08± 0.5	47.9±0.4	7.67±0.4	9.99±0.5	7.47±0.7
	Shoot	46.07±0.2	Nil	2.35±0.3	33.54±0.3	4.78±0.2	Nil	23.08±0.3	5.29± 0.3	50.01±0.6	Nil ±0.9	4.76±0.4	33.65±0.6
	Leaves	20.29±0.3	Nil	6.12±0.4	25.32±0.7	4.99 ±0.2	214.4±0.3	15.09±0.3	3.65± 0.2	24.25±0.3	Nil ±0.9	5.54±0.3	24.54±0.3
	Total	115.32±0.8	0.98±0.7	9.70±1.4	101.51±1.0	15.44±0.6	589.8±0.6	95.78±1.0	14.02±1.0	122.16±1.3	7.67±2.2	20.29±1.2	65.70±1.6
	Accumulation pattern	R>S>L	BLD	L>R>S	R>S>L	R>L>S	R>S	R>S>L	S>R>L	S>R>L	BLD	R>L>S	S>L>R
<i>S. hispidosa</i>	Root	17.03±0.5	7.00±0.0	Nil	16.65±0.6	0.57±0.4	477.2±0.6	10.23±0.8	25.46±0.8	8.76±0.7	5.67±0.7	20.35±0.3	4.65±0.1
	Shoot	14.07±0.2	Nil	Nil	45.76±0.5	Nil ±0.3	156.3±0.4	17.76±0.7	7.76±0.7	15.07±0.5	Nil	Nil	45.76±0.7
	Leaves	9.66±0.1	18.98±0.3	Nil	14.65±0.5	Nil ±0.3	245.5±0.5	25.45±0.4	3.45±0.5	7.98±0.1	20.98±0.9	Nil	15.54±0.8
	Total	40.76±0.8	25.98±0.3	Nil	77.06±1.6	0.57±1.0	879.0±1.5	53.44±1.9	36.67±2.0	31.81±1.3	26.65±1.6	20.35±0.3	65.95±1.6
	Accumulation pattern	R>S>L	L>R	BLD	S>R>L	BLD	R>L>S	L>S>R	R>S>L	S>L>R	L>R>	BLD	S>L>R
<i>P. murex L</i>	Root	20.98±0.4	10.76±0.5	2.43±0.7	18.21±0.2	8.43±0.7	45.25±0.5	37.98±0.4	22.98±0.7	25.65±0.5	20.54±0.9	15.92±0.8	4.34±0.5
	Shoot	18.74±0.5	2.32±0.7	2.54±0.4	22.54±0.5	13.65±0.7	29.33±1.4	18.76±0.5	17.78±0.7	4.50±0.6	15.87±0.5	15.94±0.9	6.74±0.5
	Leaves	22.43±0.5	9.98±0.6	5.66±0.6	6.97± 0.3	9.35± 0.7	23.78±0.5	20.34± 0.8	9.65± 0.7	18.56±0.6	14.71±0.7	15.44±0.5	7.76±0.5
	Total	62.15±1.4	23.06±1.8	10.63±1.7	47.72±1.0	31.43±2.1	98.36±2.4	77.08±1.7	50.41±2.1	49.71±1.7	51.12±2.1	47.30±2.2	18.84±1.5
	Accumulation pattern	R>S>L	L>R>S	L>R>S	S>R>L	S>L>R	R>S>L	R>L>S	R>S>L	R>S>L	R>S>L	S>L>R	L>S>R
<i>M. daigica</i>	Root	20.97±0.5	9.56±0.3	2.23±0.2	14.43±0.4	8.23± 0.3	45.67±0.5	8.76± 0.4	19.76 ±0.4	7.99± 0.5	15.87±0.5	17.34±0.6	13.54±0.5
	Shoot	14.38±0.5	2.89±0.2	2.54±0.3	23.45±0.6	14.56±0.5	33.98±0.4	19.65±0.5	5.23±0.5	6.99± 0.7	13.98± 0.5	15.98±0.4	12.76±0.4
	Leaves	20.09±0.5	8.43±0.3	8.56±0.4	9.98± 0.2	9.98± 0.4	21.76±0.5	9.07± 0.5	9.54± 0.3	5.76± 0.4	13.76± 0.2	13.43± 0.4	12.43±0.4
	Total	55.44±1.5	20.88±0.8	13.35±0.9	47.86±1.2	32.77± 1.2	101.41±1.4	37.48±1.4	34.53±1.2	20.74±1.6	43.61±1.2	46.75±1.4	38.73±1.3
	Accumulation pattern	R>L>S	R>L>S	L>R>S	S>L>R	S>L>R	R>S>L	S>L>R	R>S>L	R>S>L	R>S>L	R>S>L	R>L>S

All the values are mean of three replicates (n=3) ±standard deviation (SD), BDL: Below detection limit, R: Root, S: Shoot, L: Leaf

Table 4. BCF and TF of different Heavy metal accumulation (mg kg^{-1} DW) by various hyperaccumulator plant of a different part in the root, shoot and leaves on distillery sludge bed.

Native hyperaccumulator plants	Bioconcentration factor										
	Mn	Pb	Zn	Cr	Fe	Cu	Ni	As	Se	Mo	Co
<i>Achy. aspera</i>	81.33	17.45	17.65	18.72	15.65	94.34	1.698	1.534	15.768	15.376	12.375
<i>Amar. viridis</i>	13.75	15.75	8.021	19.75	5.675	57.92	6.254	6.124	12.751	7.101	09.995
<i>Basella alba</i>	55.4	48.96	8.765	15.06	6.111	46.98	5.121	9.123	17.232	51.121	7.100
<i>Sesbania bispinosa</i>	47.76	38.10	5.212	24.10	4.989	24.98	5.090	2.457	7.369	12.355	11.846
<i>Pedaliium murex</i> L.	14.96	10.91	5.986	18.98	3.987	22.10	6.987	5.139	9.246	7.615	10.041
<i>Momordica dioica</i>	21.05	18.04	11.05	23.55	9.098	55.20	8.989	7.410	8.789	8.355	3.356
	Translocation factor										
	Mn	Pb	Zn	Cr	Fe	Cu	Ni	As	Se	Mo	Co
<i>Achy. aspera</i>	11.55	6.355	8.543	9.871	15.64	37.15	0.009	3.33	3.335	6.324	5.435
<i>Amar. viridis</i>	3.908	3.432	8.654	7.324	14.99	42.55	1.111	6.23	4.463	7.369	7.765
<i>Basella alba</i>	3.999	9.123	9.987	3.776	14.90	28.39	1.123	3.98	7.714	7.451	13.43
<i>Sesbania bispinosa</i>	5.001	8.798	5.111	7.324	13.17	18.43	2.232	9.23	9.865	8.254	58.65
<i>Pedaliium murex</i> L.	6.012	3.432	7.213	8.654	12.44	13.44	1.989	12.45	11.345	10.23	15.65
<i>Momordica dioica</i>	9.21	7.987	9.111	9.266	19.23	20.45	0.009	7.23	14.665	9.654	16.87

4. Conclusion

The analysis of sugarcane molasses-based distillery waste revealed the presence of various organic compounds as residual pollutants; many of them compounds were known as mutagenic and carcinogenic compounds. The presence of various metals could increase the vulnerability of the flora due to the high binding tendency of metals with organic polymers. But the luxuriant growth of the native plants indicated potentiality for metal accumulation and phytoremediation. The BCF and TF analyses of these plants showed hyperaccumulation properties. Various heavy metals i.e. Pb, Cd, Ni, As, Cr, and Mo, were accumulated in leaves and shoots of *A. aspera* L., *B. alba* L. and *M. doica*, which are well known diurnal plants. Further, the transmission electron microscopy (TEM) analysis of root tissues revealed the deposition of metal granules in the root cytoplasm and cell wall of all the tested plants, which further confirmed their hyperaccumulation properties. Despite their potential in phytoremediation of organometallic pollutants, arbitrary application and subsequent harvesting of these plants are not recommended due to their functions as medicine or food crops. However, these plants can be used for the eco-restoration of sites polluted by organometallic industrial waste.

CRedit authorship contribution statement

Sonam Tripathi: Writing - original draft. **Pooja Sharma:** Kshitij Singh- Writing - original draft. **Diane Purchase:** Conceptualization, Writing - review & editing. **Ram Chandra:** Conceptualization, Visualization, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research is financially supported by the Department of Biotechnology (DBT), Govt of India. Vide Letter No. BT/PR13922/ BCE/8/1129/2015. Instrumentation facilities for SEM, FTIR, and EDX analysis from USIC, BBAU Lucknow, Uttar Pradesh, India are gratefully acknowledged. While the HPLC and GC–MS analysis from CSIR-Indian institute of toxicology research (IITR), Lucknow are highly acknowledged. TEM analysis have been conducted at SAIF facilities, AIIMS, New Delhi. The authors gratefully acknowledge all the anonymous reviewers and Editor-In-Chief for their valuable comments on the earlier version of this paper. All authors have read and agreed to the final version of the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.eti.2021.101434>.

References

- AIDA All India Distillers Association and Sugar Technology Association of India Annual Report 2004
- American Public Health Association (APHA), 2012. Standard methods for the examination of water and wastewater, method 9221 B.
- Annan, K., Dickson, R.A., Amponsah, I.K., Nooni, I.K., 2013. The heavy metal contents of some selected medicinal plants were sampled from different geographical locations. *Pharmacogn. Res.* 5 (2), 103.
- AOAC, 2002. Official methods of analysis of AOAC international. In: AOAC International, Gaithersburg.
- Bharagava, R.N., Chandra, R., Rai, V., 2008. Phytoextraction of trace elements and physiological changes in Indian mustard plants (*Brassica nigra* L.) grown in post methanated distillery effluent (PMDE) irrigated soil. *Bioresour. Technol.* 99 (17), 8316–8324.
- Blöcher, C., Dorda, J., Mavrov, V., Chmiel, H., Lazaridis, N.K., Matis, K.A., 2003. Hybrid flotation-membrane filtration process for the removal of heavy metal ions from wastewater. *Water Res.* 37 (16), 4018–4026.
- Bose, S., Bhattacharyya, A.K., 2008. Heavy metal accumulation in wheat plants grown in soil amended with industrial sludge. *Chemosphere* 70 (7), 1264–1272.
- Bratskaya, S.Y., Pestov, A.V., Yatluk, Y.G., Avramenko, V.A., 2009. Heavy metals removal by flocculation/precipitation using N-(2-carboxyethyl) chitosans. *Colloids Surf. A* 339 (1–3), 140–144.
- Chandra, R., Bharagava, R.N., Rai, V., 2008. Melanoidins as a major colorant in sugarcane molasses-based distillery effluent and its degradation. *Bioresour. Technol.* 99 (11), 4648–4660.
- Chandra, R., Bharagava, R.N., Yadav, S., Mohan, D., 2009. Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. *J. Hard Mater.* 162 (2–3), 1514–1521.
- Chandra, R., Kumar, V., Tripathi, S., Sharma, P., 2018. 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. *Ecol. Eng.* 111, 143–156.
- Chandra, R., Yadav, S., Yadav, S., 2017. Phytoextraction potential of heavy metals by native wetland plants growing on chlorolignin containing sludge of pulp and paper industry. *Ecol. Eng.* 98, 134–145.
- Cheraghi, M., Lorestani, B., Khorasani, N., Yousefi, N., Karami, M., 2011. Findings on the phytoextraction and phytostabilization of soils contaminated with heavy metals. *Biol. Trace. Elem. Res.* 144 (1–3), 1133–1141.
- Dialynas, E., Diamadopoulos, E., 2009. Integration of a membrane bioreactor coupled with reverse osmosis for advanced treatment of municipal wastewater. *Desalination* 238 (1–3), 302–311.
- Duthie, J.F., Parker, R.N., Turrill, W.B., 1903. Flora of the upper Gangetic plain, and the adjacent Siwalik and sub-Himalayan tracts (Vol. 2). In: Office of the Superintendent of Government Print.

- Franchi, E., Rolli, E., Marasco, R., Agazzi, G., Borin, S., Cosmina, P., Petruzzelli, G., 2017. Phytoremediation of a multi contaminated soil: mercury and arsenic phytoextraction assisted by mobilizing agent and plant growth-promoting bacteria. *J. Soil. Sediment.* 17 (5), 1224–1236.
- Ghassemzadeh, F., Yousefzadeh, H., Arbab-Zavar, M.H., 2008. Arsenic phytoremediation by *Phragmites australis*: green technology. *Int. J. Environ. Studies* 65 (4), 587–594.
- Gupta, A.K., Sinha, S., 2006. Chemical fractionation and heavy metal accumulation in the plant of *Sesamum indicum* (L.) var. T55 was grown on soil amended with tannery sludge: Selection of single extractants. *Chemosphere* 64 (1), 161–173.
- Gupta, A.K., Sinha, S., 2007. Phytoextraction capacity of the plants growing on tannery sludge dumping sites. *Bioresour. Technol.* 98 (9), 1788–1794.
- Gupta, A.K., Sinha, S., 2008. Decontamination and/or revegetation of fly ash dykes through naturally growing plants. *J. Hazard. Mater.* 153 (3), 1078–1087.
- Hadi, F., Bano, A., Fuller, M.P., 2010. The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations. *Chemosphere* 80 (4), 457–462.
- Jaison, S., Muthukumar, T., 2017. Chromium accumulation in medicinal plants growing naturally on tannery contaminated and non-contaminated soils. *Biol. Trace Elem. Res.* 175 (1), 223–235.
- Kansal, A., Rajeshwari, K.V., Balakrishnan, M., Lata, K., Kishore, V.V.N., 1998. Anaerobic digestion technologies for energy recovery from an industrial wastewater-a study in the Indian context. *TERI Inf. Monit. Environ. Sci.* 3 (2), 67–75.
- Kumar, N., Baudh, K., Kumar, S., Dwivedi, N., Singh, D.P., Barman, S.C., 2013. Accumulation of metals in weed species grown on the soil contaminated with industrial waste and their phytoremediation potential. *Ecol. Eng.* 61, 491–495.
- Kumar, A., Dhall, P., Kumar, R., 2010. Redefining BOD: COD ratio of pulp mill industrial wastewaters in BOD analysis by formulating a specific microbial seed. *Int. Biodeterioration Biodegrad.* 64 (3), 197–202.
- Laghlimi, M., Baghdad, B., El Hadi, H., Moussadak, R., Taoufik, M., 2015. Assessment of heavy metals soil contamination in the abandoned Pb-mine of Zaida (Morocco). *Eur. J. Sci. Res.* 129 (2), 167–178.
- Liu, X., Zhu, H., Qin, C., Zhou, J., Zhao, J.R., Wang, S., 2013. Adsorption of heavy metal ion from aqueous single metal solution by aminated epoxy-lignin. *BioResources* 8 (2), 2257–2269.
- Ma, Y., Prasad, M.N.V., Rajkumar, M., Freitas, H., 2011. Plant growth-promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. *Biotech. Adv.* 29 (2), 248–258.
- Mazumdar, K., Das, S., 2015. Phytoremediation of Pb, Zn, Fe, and Mg with 25 wetland plant species from a paper mill contaminated site in North East India. *Environ. Sci. Pollut. Res.* 22 (1), 701–710.
- Migo, V.P., Del Rosario, E.J., Matsumura, M., 1997. Flocculation of melanoidins induced by inorganic ions. *J. Ferment. Bioengineer.* 83 (3), 287–291.
- Najeeb, U., Jilani, G., Ali, S., Sarwar, M., Xu, L., Zhou, W., 2011. Insights into cadmium induced physiological and ultra-structural disorders in *Juncus effusus* L. and its remediation through exogenous citric acid. *J. Hard Mater.* 186 (1), 565–574.

- Noor, E.A., Al-Moubaraki A.H. Thermodynamic study of metal corrosion and inhibitor adsorption processes in mild steel/1-methyl-4 [4(-x)-styryl pyridinium iodides/ hydrochloric acid systems Mater. Chem. Phys. 110 1 2008 145 154
- Ott, L., 1984. An Introduction to Statistical Methods and Data Analysis. second ed. PWS, Boston, MA.
- Pavel P.B., Puschenreiter M., Wenzel W.W., Diacu E. Barbu C.H., 2014. Aided phytostabilization using *Miscanthus sinensis giganteus* on heavy metal-contaminated soils. Sci. Total Environ. 479 2014 125 131
- Rajkumar, M., Sandhya, S., Prasad, M.N.V., Freitas, H., 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotech. Adv. 30 (6), 1562–1574.
- Sahi, S.V., Bryant, N.L., Sharma, N.C., Singh, S.R., 2002. Characterization of a lead hyperaccumulator shrub, *Sesbania drummondii*. Environ. Sci. Technol. 36 (21), 4676–4680.
- Saraf, A., Samant, A., 2013. Evaluation of some minerals and trace elements in *Achyranthes aspera* Linn. Int. J. Pharma Sci. 3 (3), 229–233.
- Sellami, F., Hachicha, S., Chtourou, M., Medhioub, K., Ammar, E., 2008. Maturity assessment of composted olive mill wastes using UV spectra and humification parameters. Bioresour. Technol. 99 (15), 6900–6907.
- Sessitsch, A., Kuffner, M., Kidd, P., Vangronsveld, J., Wenzel, W.W., Fallmann, K., Puschenreiter, M., 2013. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol. Biochem. 60, 182–194.
- Shammi, M., Kashem, M.A., Rahman, M.M., Hossain, M.D., Rahman, R., Uddin, M.K., 2016. Health risk assessment of textile effluent reuses as irrigation water in leafy vegetable *Basella alba*. Int. J. Recycl. Org. Waste Agric. 5 (2), 113–123.
- Sharma, P., Pandey, A.K., Udayan, A., Kumar, S., 2021a. Role of microbial community and metal-binding proteins in phytoremediation of heavy metals from industrial wastewater. Bioresour. Technol. 124750.
- Sharma, P., Purchase, D., Chandra, R., 2021b. Residual pollutants in treated pulp paper mill wastewater and their phytotoxicity and cytotoxicity in *Allium cepa*. Environ. Geochem. Health 2021 Jan 5. doi: 10.1007/s10653-020-00730-z
- Sharma, P., Rath, S.K., 2020. Potential applications of fungi in the remediation of toxic effluents from pulp and paper industries. In: Fungi Bio-Prospects in Sustainable Agriculture Environment and Nanotechnology. Academic Press, pp. 193–211.
- Sharma, P., Singh, S.P., Pandey, S., Thanki, A., Singh, N.K., 2020a. Role of potential native weeds and grasses for phytoremediation of endocrine-disrupting pollutants discharged from pulp paper industry waste. Bioremediation Pollut. 17–37.
- Sharma, P., Tripathi, S., Chandra, R., 2020b. Metagenomic analysis for profiling of microbial communities and tolerance in metal-polluted pulp and paper industry wastewater. Bioresour. Technol. 124681.
- Sharma, P., Tripathi, S., Vadakedath, N., Chandra, R., 2020c. In-situ toxicity assessment of pulp and paper industry wastewater on *Trigonella foenum-graecum* L: Potential source of cytotoxicity and chromosomal damage. Environ. Technol. Innov. 101251.

- Singh, A., Prasad, S.M., 2014. Effect of an agro-industrial waste amendment on cd uptake in *Amaranthus caudatus* grown under contaminated soil: an oxidative biomarker response. *Ecotoxicol. Environ. Safety* 100, 105–113.
- Singh, H., Verma, A., Kumar, M., Sharma, R., Gupta, R., Kaur, M., Sharma, S.K., 2017. Phytoremediation: a green technology to clean up the sites with low and moderate levels of heavy metals. *Austin. Biochem.* 2 (2), 1012.
- Singh, S., Zacharias, M., Kalpana, S., Mishra, S., 2012. Heavy metals accumulation and distribution patterns in different vegetable crops. *J. Environ. Chem. Ecotoxicol.* 4 (10), 170–177.
- Sinha, S., Gupta, A.K., Bhatt, K., 2007. Uptake and translocation of metals in fenugreek grown on soil amended with tannery sludge: involvement of antioxidants. *Ecotoxicol. Environ. Safety* 67 (2), 267–277.
- Sushil, S., Batra, V.S., 2006. Analysis of fly ash heavy metal content and disposal in three thermal power plants in India. *Fuel* 85 (17–18), 2676–2679.
- Tong, Y.P., Kneer, R., Zhu, Y.G., 2004. Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. *Trends Plant Sci.* 9 (1), 7–9.
- USEPA, 2012. Endocrine Disruptor Screening Program. Universe of Chemicals.
- Vijaya Kumar, S., Sankar, P., Varatharajan, R., 2009. Anti-inflammatory activity of roots of *Achyranthes aspera*. *Pharm. Biol.* 47 (10), 973–975.
- WHO, World Health Organization, 1978. International Classification of Procedures in Medicine (Vol. 2). World Health Organization.
- Yadav, S., Chandra, R., 2018. Detection and assessment of the phytotoxicity of residual organic pollutants in sediment contaminated with pulp and paper mill effluent. *Environ. Monit. Assess.* 190 (10), 581.
- Yoon, J., Cao, X., Zhou, Q., Ma, L.Q., 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.* 368 (2–3), 456–464.