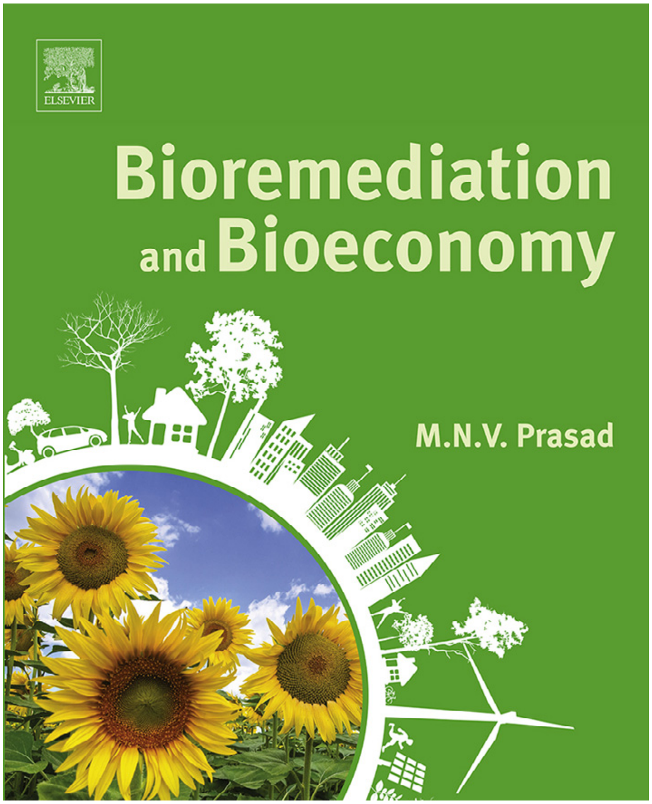

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CHAPTER

12

CULTIVATION OF SWEET SORGHUM ON HEAVY METAL-CONTAMINATED SOILS BY PHYTOREMEDIATION APPROACH FOR PRODUCTION OF BIOETHANOL

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1 INTRODUCTION

The term *heavy metal* (HM) has a wide range of meanings, and there has been no consistent definition by any authoritative body such as International Union of Pure and Applied Chemistry (IUPAC) over the past 60 years (Duffus, 2002). But over the past 2 decades, this term has been used by numerous publications and legislations for indicating a group name for metals or semimetals that cause human, phyto, animal, and also ecotoxicity. Though the imprecise term is defined by several researchers at various levels including density, atomic number, atomic weight, chemical properties, and toxicity, there is no connectivity between these properties. Since this chapter deals with bioremediation aspects, HMs causing human and ecotoxic effects were considered further. Three kinds of HMs are of concern, including toxic metals (Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (Pd, Pt, Ag, Au, Ru, etc.), and radionuclides (U, Th, Ra, Am, etc.) (Wang and Chen, 2006).

The stability and nondegradability of metals mean higher exposure of HMs to humans and animals, and numerous reports are available for the related health and ecological issues (Caussy et al., 2003; Li et al., 1995). Toxic effects of HMs may be chronic or acute, which depends on the route of transfer and reactive forms. For instance, for Cd, all forms are toxic; for Pb, organic forms are highly toxic; for As, inorganic arsenate [As(+5)] or [As(+3)] is highly toxic; for Hg and Hg(II), organomercurials, mainly methylmercury (Mudgal et al., 2010). Toxic effects of these major HMs are periodically reviewed by many researchers (Burbacher et al., 1990; Duruibe et al., 2007; Wongsasuluk et al., 2014; Zatta, 2001). Representatives of HM-related health issues and their tolerable limits are summarized in Table 1.

The HMs are sourced from both natural and anthropogenic activities (Chopra et al., 2009; Li et al., 2009). Parent rocks are the natural contributors, and their HM content is usually found to be low

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Table 1 Effect of HMs on Humans

HMs	Effect on Human Health	JECFA Tolerable Limits (x/kg bw/day)
As	Bronchitis, dermatitis, poisoning	2.1 μg
Cd	Renal dysfunction, bone defects, blood pressure, bronchitis, cancer	25 μg
Pb	Mental retardation in children, developmental delay, fatal infant encephalopathy, congenital paralysis, sensor neural deafness, epilepsy, acute/chronic damage of CNS, liver, kidney, and GI tract	0.025 μg
Hg	Tremors, gingivitis, psychological changes, acrodynia, spontaneous abortion, protoplasm poisoning, CNS damage	4 μg
Zn	CNS damage, corrosive effects on skin	0.3-1 mg
Cr	CNS damage, fatigue, irritability	–
Cu	Anemia, liver and kidney damage, stomach and intestinal irritation	0.5 mg

JECFA, Joint FAO/WHO Expert Committee on Food Additives.
Source: JECFA (1982, 2011, 2013).

depending on the parent rock composition. Various anthropogenic activities transfer the HMs through air, water, and soil, the major transmitters of any kind of pollutants. Such routes and sources are (1) air, which has mining, smelting, and refining of fossil fuels; smoke from production units of metallic goods; and vehicular exhaust; (2) water, having domestic and industrial sewage and effluents, thermal power plants, and atmospheric fallout; and (3) soil having agricultural and animal wastes, municipal and industrial sewage, coal ashes, fertilizers, discarded metal goods, and atmospheric fallout. Anthropogenic sources are the maximum contributors for metals rather than natural sources (Nriagu and Pacyna, 1988). A recent study by Millward and Turner (2001) also states that, anthropogenic factors are the major metal contributors as they alter the natural biogeochemical cycles.

According to the Environmental Protection Agency (EPA) report, the United States has more than 40,000 contaminated sites as of May 2004. In addition, 100,000 ha of cropland, 55,000 ha of pasture, and 50,000 ha of forest have been lost by HM contamination and demands for reclamation (McGrath et al., 2001; Ragnarsdottir and Hawkins, 2005). In Europe, around 2 million sites were contaminated with HMs, cyanide, mineral oil and chlorinated hydrocarbons (EEA, 2005). In developing countries, particularly in India, China, Pakistan, and Bangladesh, HM pollution occurs by the release of untreated industrial effluents into the surface drains, which further spreads to agricultural lands. Untreated effluent is sometimes used for irrigation due to water scarcity, where it acts as an HM source for agricultural croplands (Ragnarsdottir and Hawkins, 2005). In addition, most agricultural land has been used for construction purposes. All these factors together have led to shrinkage of healthy agricultural cropland. The increasing demand for lands has forced farmers to use contaminated sites for crop cultivation. This chapter deals with remediation of HM-contaminated sites, specifically by phytoremediation, and the role of phytoremediation in improving the economy.

2 REMEDIATION MEASURES FOR HMs

Many remediation techniques involving physical (soil replacement, thermal desorption) and chemical (leaching and fixation) methods, along with use of a broad range of chemical additives, are available for HM removal from soil (Bricka et al., 1993; Yao et al., 2012). Due to the difficulties involving scale-up processing, adaptability, site conditions, low efficiency, loss of soil structure and fertility, metal specificity, and cost-effectiveness, they have not been promoted on a large scale. Figures 1 and 2 depict various physical and chemical remediation measures with their pros and cons.

The phytotoxic effects of HMs include reduction in plant growth and protein content, loss of mineral homeostasis, and hence loss in yield and crop quality (Chibuike and Obiora, 2014). Yet some plant species are able to tolerate the negative effects of HM in addition to having the ability to extract in their tissues. This paves a way for the development of a technology called phytoremediation, also known as botanical bioremediation or green remediation, which involves the use of plant species for the extraction, removal, sequestration, detoxification, immobilization of toxic substances through various mechanisms. This also overcomes the negative effects associated with the physical and chemical remediation methods mentioned earlier. Phytoremediation can be applied for different matrixes such as soil, water, and sediment. All these aspects have been summarized by many reviews from past decades to the current scenario (Chaney et al., 1997; Gomes, 2012; Moffat, 1995; Salt et al., 1977; Sharma and Pandey, 2014).

Besides the chemical states of HMs, there are different physical forms: (a) dissolved (in soil solution), (b) exchangeable (organic and inorganic components), (c) as structural components of the lattices of soil minerals, and (d) as insoluble precipitates with other soil components. The former two forms are available to the plants. The latter two forms remain for a long term (Aydinalp and Marinova, 2003). Depending on the physical and chemical states of HMs, phytoremediation in soil occurs through any of the following modes: phytoextraction, phytostabilization, and phytovolatilization (Sharma and Pandey, 2014), which is depicted in Figure 3.

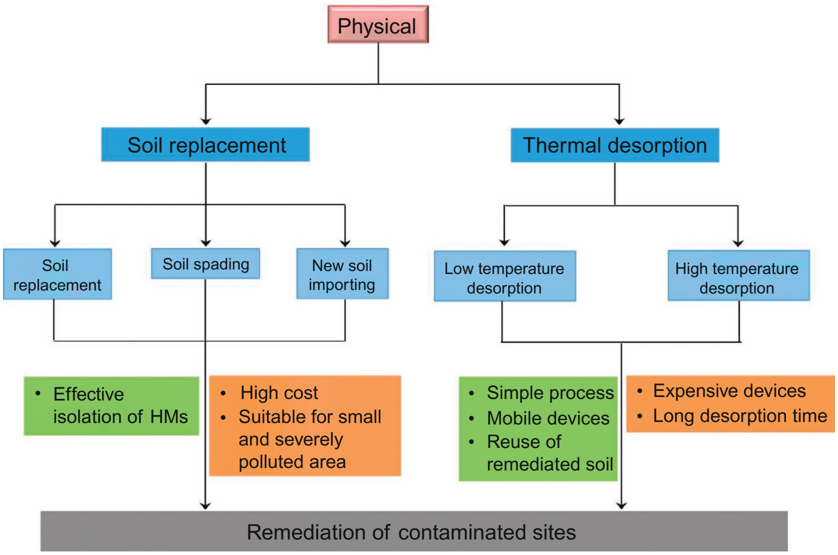

FIGURE 1

Physical remediation measures for HM removal. Strategies/technologies involved for HM remediation are in blue shapes. Positive impacts and risk factors associated with the strategies have been indicated in green and orange shapes respectively.

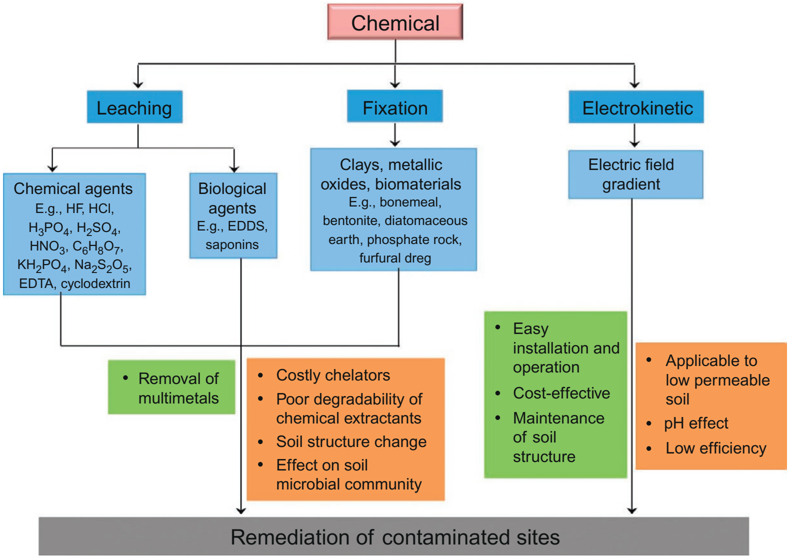

FIGURE 2

Chemical remediation measures for HM removal. Strategies/chemicals involved for HM remediation are in blue shapes. Positive impacts and risk factors associated with the strategies have been indicated in green and orange shapes respectively.

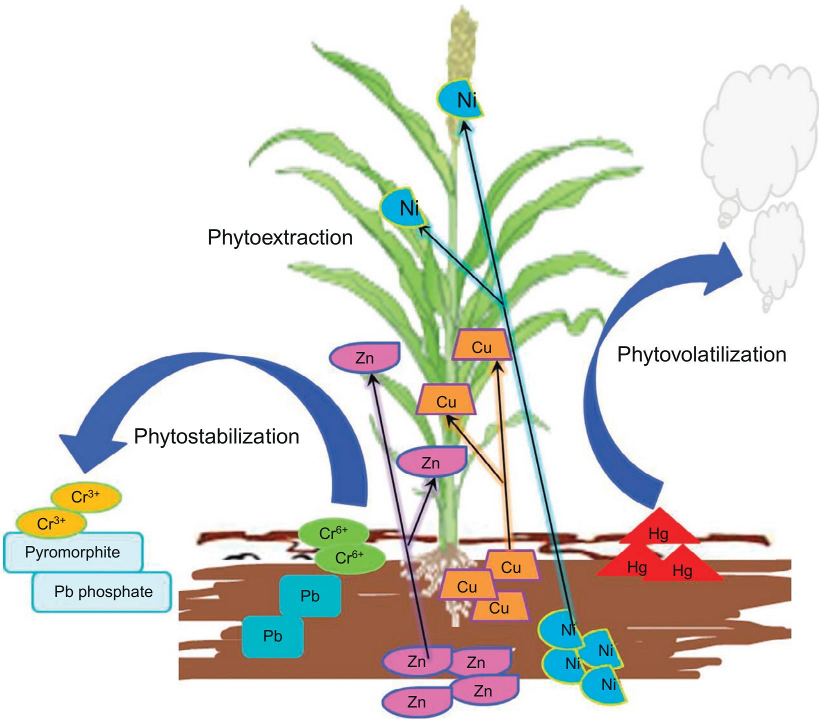

FIGURE 3

HM removal mechanisms in soil by phytoremediation.

Phytoextraction: Also known as phytoaccumulation, in phytoextraction the HMs in soil are transferred to the above-ground biomass such as the shoots and leaves with the aid of roots, and through the process of absorption, concentration, and precipitation. Most of the HMs including Ni (Kukier and Chaney, 2004), Cu (Delorme et al., 2001), and Zn (Sun et al., 2010) have been extracted by this mode.

Phytostabilization: In phytostabilization, toxic forms of HMs are changed into nontoxic or less toxic forms and reduce the bioavailability. The HMs are absorbed or accumulated in roots or precipitated into the root zone, either naturally or by chemical amendments. Since this process is confined to the rhizosphere, migration of HMs can be inhibited, which helps in conserving ground- and surface water and reduces bioavailability of metal into the food chain. Many HMs including As, Pb, Cd, Cr, Cu, and Zn can be stabilized by this mode (Brennan and Shelley, 1999). Still, it has some drawbacks such as contaminant remaining in soil and requirement for extensive fertilizers and soil amendments application (Sharma and Pandey, 2014).

Phytovolatilization: Phytovolatilization involves the transformation of HMs into volatile forms through leaves, which are further transpired into atmosphere. Hg is the major HM, phytoremediated by phytovolatilization (Rugh et al., 1996). But the negative impact associated with Hg is that recycling by precipitation and redeposition of atmospheric Hg into lakes and oceans leads to production of methylmercury.

A strengths, weaknesses, opportunities, and threats analysis done on phytoremediation by Gomes (2012) revealed that phytoremediation has its own pros and cons as do physical and chemical remediation measures. Major weaknesses observed were (i) plant selection with requirement of multitraits such as fast growth, high biomass, deep roots, and easy harvesting; (ii) slow process; and (iii) limited practical experience. Though phytoremediation has these weaknesses, it has many supporting strengths such as (i) high public acceptance; (ii) maintenance of soil biological components; (iii) environmental benefits such as control of soil erosion, carbon sequestration, and creation of wildlife habitat; (iv) generation of recyclable metal-rich plant tissues; (v) socioeconomic benefits via local labor employment and buildup of value-added industry; (vi) cost-effectiveness; and (vii) sustainability.

3 PHYTOREMEDIATION OF HMs: HYPERACCUMULATORS

Plant selection is a crucial first step for phytoremediation. It is already known that the elements present in the soil will be reflected to some extent in the plants, this extent represented by concentration, a platform for differentiating and selecting a plant for phytoremediation purpose. The term *hyperaccumulators* was initially used by Jaffré et al. (1976) to indicate higher metal uptake potential (25% on Dry Matter (DM)) of *Sebertia acuminata*. It was further used by Brooks et al. (1977) for describing plants whose dried tissues have $>1000\,\mu g\,g^{-1}$ Ni; they proposed this is the discriminatory concentration threshold for differentiating normal plants and hyperaccumulating plants. Jaffré (1980) has refined the nomenclature by using the terms *hypermanganesophores* and *hypernickelophores* to describe metal extraction specificity of the plants. But the public exposure toward the use of hyperaccumulating plants for HM removal has increased in later years (Chaney, 1983; Anonymous, 1990). The necessary features that distinguish hyperaccumulators from nonhyperaccumulating plant species include higher rate of (i) HM uptake, (ii) root-to-shoot translocation, and (iii) detoxification and sequestration of HM (Rascio and Navari-Izzo, 2011).

Baker and Brooks (1989) reviewed the distribution of terrestrial plants with hyperaccumulating potential and found 145 hyperaccumulators for Ni with a distribution in 6 suborders, 17 orders, and 22 families including herbs, shrubs, and trees. This indicates that hyperaccumulators are not closely related, but they possess the common feature of growth on metalliferous soils without phytotoxic effects (Rascio and Navari-Izzo, 2011). A recent review by van der Ent et al. (2012) summarized several criteria used for hyperaccumulation threshold and suggested concentration criteria for different metals based on critical evaluation of numerous hyperaccumulation reports: Cd, Se, and Tl—100 $\mu g\, g^{-1}$; Co, Cu, and Cr—300 $\mu g\, g^{-1}$; Ni, Pb, and As—1000 $\mu g\, g^{-1}$; Zn—3000 $\mu g\, g^{-1}$; Mn—10,000 $\mu g\, g^{-1}$. This evaluation also identified more than 500 plant taxa that can be categorized as hyperaccumulators for one or more elements including Asteraceae, Brassicaceae, Caryophyllaceae, Cunouniaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, and Violaceae. The most-researched studies of hyperaccumulation model systems include *Thlaspi* sp., (Delorme et al., 2001; Idris et al., 2004), *Brassica* sp., (Quartacci et al., 2014; Wu et al., 2013), and *Alyssum* sp. (Barzanti et al., 2011; Bayramoglu et al., 2012). But features found in these plant families such as slow growth, shallow root system, small biomass, and unknown agronomic potential of hyperaccumulators have made it necessary to find alternative plant species for phytoremediation.

4 PHYTOREMEDIATION OF HMs: ENERGY CROPS

Various research groups differ on plant selection for phytoremediation. Many research groups have suggested that the use of hyperaccumulators for HM remediation is of prime importance rather than biomass (Chaney et al., 1997; van der Ent et al., 2012). However, the success of phytoremediation depends not only on the complete removal of toxic substances but also on the generation of valuable biomass including timber, bioenergy, feedstock for pyrolysis, and biofortified products or ecologically important species in order to demonstrate cost-effectiveness (Conesa et al., 2012; van der Lelie et al., 2001). Report of Meers et al. (2010) strongly supports the use of bioenergy crops for HM phytoremediation. Maize was tested under field conditions in Flanders, Belgium, in soil contaminated with HMs, Pb, Cd, Zn, and As by historic smelter activities. They stated that cultivation of energy maize in this region could result in the production of 30,000-42,000 kW h including electrical and thermal renewable energy per hectare. This could replace a coal-fed power plant with the reduction of up to 21 tons ha^{-1} $year^{-1}$ CO_2 along with the HM removal.

Energy crops fall into two categories: annuals: sweet sorghum and fiber sorghum, kenaf, and rapeseed; and perennials, a category further subdivided into (a) agricultural: wheat, sugar beet, cardoon, reeds, miscanthus, switchgrass, and canary reed grass; and (b) forest: willows, poplars, eucalyptus, and black locust (Simpson et al., 2009). High biomass crops with HM tolerance such as Indian mustard, oat, maize, barley, sunflower, ryegrass, fast-growing willow, and poplars have been studied (Komárek et al., 2007; Meers et al., 2005; Shen et al., 2002; Vervaeke et al., 2003). Table 2 summarizes the HM removal efficiency of several agricultural crops (Zhuang et al., 2009). In conclusion, knowledge of energy crop cultivation under contaminated conditions will provide new avenues for bioeconomy and also for reclamation of contaminated soils.

5 PHYTOREMEDIATION OF HMs BY SUGAR CROPS: SWEET SORGHUM

Among the energy crops, sugar crops (sugarcane, sugar beet, and sweet sorghum) with HM remediation capacity have a great impact on the bioenergy-bioethanol (Yadav et al., 2011). Reports of Rayment et al. (2002), Yadav et al. (2010), and Jain et al. (2010) on sugarcane reveals their HM accumulation

Table 2 Comparison of HM Removal Efficiency by Agricultural Crops

Crop	Binomial Name	Biomass (tons ha^{-1})	Heavy Metal Uptake (kg ha^{-1})				References
			Pb	Cd	Zn	Cu	
Sorghum	*Sorghum bicolor*	25.8 (dw)	0.35	0.052	1.44	0.24	Zhuang et al. (2009)
		22.1 (dw)	0.38	0.006	1.22	0.64	Marchiol et al. (2007)
Sunflower	*Helianthus annuus*	–	0.091	0.002	0.41	0.12	Marchiol et al. (2007)
		24 (dw)	0.016	–	2.14	–	Madejón et al. (2003)
Indian mustard	*Brassica juncea*	7.3 (dw)	–	0.007	0.89	0.15	Keller et al. (2003)
Tobacco	*Nicotiana tabacum*	12.6 (dw)	–	0.042	1.83	0.47	
Alfalfa	*Medicago sativa*	45.9 (ww)	0.115	0.013	0.438	0.124	Ciura et al. (2005)
Maize	*Zea mays*	92.7 (ww)	0.042	0.0093	0.45	0.096	
Barley	*Hordeum vulgare*	5.84 (dw)	0.035	0.014	1.07	0.057	Soriano and Fereres (2003)

dw, dry weight; ww, wet weight.
Source: Zhuang et al. (2009).

potential for Cd, Zn, and Hg. Sugarcane grown near the municipal landfill site and medical waste treatment system in Brazil is also found to accumulate the HM such as Cd, Cr, Cu, Hg, Mn, Pb, and Zn (Segura-Muñoz et al., 2006). A study on HM content of vegetables in Pakistan revealed that sugar beet has the potential to accumulate Cd, Pb, As, and Hg but at the safest and legally permissible level (Abbas et al., 2010).

Sorghum (*Sorghum bicolor* L. Moench), a C4 annual grass valued for food, feed, fiber, and feedstock is known by many names such as jowar (India), kaoliang (China), great millet and guinea corn (west Africa), kafir corn (south Africa), mtama (north Africa), dura (Sudan), and milo or milo-maize (Unites States) (Purseglove, 1972). It is also known as "sugarcane of the desert" and "camel among crop" for its hardiness in drought. It can be grown in tropical, subtropical, temperate, and semiarid regions, and also in poor-quality soils (Sanderson et al., 1992). It has many salient features such as rapid growth, high sugar content (10-15%), higher biomass, wider adaptability to harsh agroclimatic conditions, and metal-absorbing property (Zhuang et al., 2009; Rao et al., 2009). Sweet sorghum is type of *S. bicolor*, generally cultivated for syrup and also forage and feed. It has sweet juicy stalks and higher quantity of sugars (both glucose and fructose) than grain sorghum; hence, it is called sweet sorghum. So, it can serve as the best candidate for playing dual role in phytoremediation and bioeconomy via bioethanol "sweet fuel" production and also related coproduct generation (Rao et al., 2012). The sweet sorghum varieties developed by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the related research work done on biofuel will be discussed later in this chapter.

The first hint of the HM absorption of sorghum was shown by An (2004) during an ecotoxic assessment, when he found sorghum can accumulate more Cd than cucumber, wheat, and sweet corn. Subsequent proof was given by Kaplan et al. (2005) during the analysis on sulfur-containing waste as a soil amendment under pot trials. It has been observed that, Ni, Cr, and Co accumulation occurs in roots, whereas Cd accumulation occurs in straw of sorghum. This indicates the efficiency of sorghum fibrous roots in HM absorption. A similar trend was observed on a short-term study on hydroponically grown *S. bicolor* for Pb, Cd, and Zn removal (Hernández-Allica et al., 2008; Soudek et al., 2012). In a 3-month microcosm study with artificially polluted soil containing Cd and Zn, sorghum was found to have 122 mg Cd kg^{-1} dry weight (DW) in shoot. This is higher than the threshold value of 100 mg kg^{-1} DW set for hyperaccumulators. From the various soil physicochemical and biological properties evaluated, it is noted that, phytoremediation with sorghum is able to recover the soil function, though the experimental soil has more phytotoxicity than the control treatments (Epelde et al., 2009).

The first *in situ* phytoremediation pilot plant was established during 2005 in Torviscosa, Italy, where Marchiol et al. (2007) designed a study to evaluate the phytoremediation effect of high biomass crops including *S. bicolor* and *H. annuus*. The experimental site possesses multimetal contaminants including As, Cd, Cu, Zn, and Fe, and it is under the national priority list of polluted sites in Italy. Limited metal extraction is observed in both the plants; however, the experimental design did not involve any practices such as soil amendments for enhancing metal bioavailability. It is understood that the factors considered for successful agriculture should also be considered for successful phytoremediation.

A study by Zhuang et al. (2009) is the first study conducted on HM remediation by sweet sorghum in a field at Lechang city, China, which has been contaminated with Pb, Cd, Zn, and Cu via atmospheric emissions and surface irrigation with mining wastewaters. The study was designed to evaluate the sweet sorghum varieties Keller, Rio, and Mary (bred for ethanol production in the United States) alone and in combination with soil amendments such as $(NH_4)_2SO_4$, NH_4NO_3, and ethylenediaminetetraacetic acid (EDTA). It was observed that there was no difference between the cultivars and biomass yield, whereas HM extraction efficiency was in the order of Keller > Mary > Rio. Among the soil amendments, EDTA promotes Pb accumulation, whereas $(NH_4)_2SO_4$ and NH_4NO_3 promote Zn and Cd accumulation. So, this assisted phytoextraction with facilitated agronomic practices serves as a sustainable remediation measure.

6 SWEET SORGHUM: A FEEDSTOCK FOR "SWEET FUEL" BIOETHANOL

Every year, fossil fuel sources are getting depleted, and they are anticipated to run out within the next 40-50 years. In addition, the consequences of fossil fuels such as global warming, acid rain, and urban smog have necessitated the shift to renewable energy sources such as biofuels, which are less harmful to the environment and also sustainable. Among the biofuels, ethanol is one of the prime alternatives. Though it has 68% lower equivalent energy than petroleum fuel, it is gaining in importance due to its complete combustion and release of less toxic by-products than other alcoholic and fossil fuels. Another contributing factor is its production from a broad range of feedstocks such as sugar (sugarcane, sugar beet, sweet sorghum), starch (corn, cassava), and lignocellulosic (agri-by-products: corn stover and fiber, wheat and barley straws, sugarcane bagasse, seed cake; woody biomass: hardwood and softwood; energy crops: switchgrass, poplar, banagrass, miscanthus, etc.) (Minteer, 2006; Vohra et al., 2014). All these factors will make ethanol the "fuel of the future," to use Henry Ford's phrase coined during his preparation of the Model T Ford. He designed the model to run on either gasoline or ethanol, with the vision of building a vehicle that was affordable for the working family and powered by a fuel that would boost the rural farm economy (Kovarik, 1998).

During 2009-2010, the world ethanol production was about 100 billion liters with consumption rates of 68% for fuel, 21% for industrial, and 11% for potable (Lichts, 2010). For fuel substitution, it is used with gasoline as either E15 (15:85%, ethanol vs. gasoline) or E85 (85:15%, ethanol vs. gasoline). Each country has set its own regulations for blending ethanol and even has set target requirements for the future (Cheng and Timilsin, 2011). In India in 2003, the Government of India (GOI) mandated the use of 5% ethanol blend in gasoline through its ambitious Ethanol Blending Program (EBP). Since 2003, the trade balance for ethanol has been generally negative. The balance has tapered down, however, from its peak of $140 million in 2005 to $11 million in 2012, indicating a gradual rise in export of ethanol and other spirits. In order to promote biofuels as an alternative energy source, the GOI in 2009 announced a comprehensive National Policy on Biofuels formulated by the Ministry of New and Renewable Energy (MNRE), calling for blending at least 20% of biofuels with diesel (biodiesel) and petrol (bioethanol) by 2017. The policies are designed to facilitate and bring about optimal development and utilization of indigenous biomass feedstock for biofuel production (Basavaraj et al., 2012).

Starch-based feedstocks contribute for higher ethanol production than sugar-based feedstocks (60 vs. 40%). But the use of starch based feedstock is limited due to higher energy requirement for its saccharification process (Vohra et al., 2014; Mussatto et al., 2010). Among the feedstocks, sugarcane is the major stock in tropical areas such as Brazil, Colombia, and India, whereas corn is the major stock in the United States, the European Union, and China (Cheng and Timilsin, 2011). Increased ethanol demand, decreased feedstock production, lack of clear technology, and the question of food/feed versus fuel have created the need for alternative feedstocks (Vohra et al., 2014).

Sweet sorghum has come to play a key role for this interlinked issue, and ethanol production from sweet sorghum is not a new process since it has 3 decades of history by various technologies (Christakopoulos et al., 1993; Kargi and Curme, 1985; Kargi et al., 1985; Lezinou et al., 1994; Mamma et al., 1995). However, it has received renewed attention for its beneficial characteristics, such as ethanol production at lower cost over sugarcane and sugar beet along with several by-products that enhance farmers' economy (Tables 3 and 4). In addition, the technology for alcoholic fermentation from sugar- and sucrose-containing feedstock is a well-known and mastered process. Some varieties of sweet sorghum have a significant sucrose content (500 gallons syrup per hectare), a major feature required for ethanol. In sweet sorghum, the sugar is stored in the main stalk, which can be recovered by pressing the stalks through rollers (similar to sugarcane processing). This yields about 20 gallons of ethanol per ton of stalks (Kojima and Johnson, 2005). Approximately, 50-85 tons ha^{-1} of sweet sorghum stalks yields 39.7-42.5 tons ha^{-1} of juice, which after fermentation produces 3450-4132 L ha^{-1} ethanol (Serna-Saldívar et al., 2012). Other studies have shown similar ethanol production levels: 3296 L ha^{-1} (Kim and Day, 2011) and 4750-5220 L ha^{-1} (Wu et al., 2010). In addition, sweet sorghum can rule out the food/feed versus fuel question due to the farmer's benefit from sorghum grains, after the stalk is harvested for juice (Kojima and Johnson, 2005). Besides this, the pressed stalk, called sweet sorghum bagasse, has several avenues in improving rural economy via ruminant/poultry feed and as raw material for biofertilizer production, paper making, and co-product generation including power (Rao et al., 2012).

Table 3 Favorable Traits of Sweet Sorghum

As Crop	As Ethanol Source	As Bagasse	As Raw Material for Industrial Products
Short duration (3-4 months)	Eco-friendly processing	High biological value	Paper and pulp making
C4 dryland crop	Less sulfur	Rich in micronutrients	Butanol, lactic acid, acetic acid production
Good tolerance to biotic and abiotic constraints	High octane ring	Ruminant/poultry feed	Alcoholic and nonalcoholic beverage production
Meets fodder and food needs	Automobile friendly (up to 25% of ethanol-petrol mixture without engine modification)	Power generation	Coproduct generation: dry ice, fuel oil, and methane
Non-invasive species		Biocompost	
Low soil N_2O and CO_2 emission		Good for silage making	
Seed propagated			

Source: *Rao et al. (2012).*

Table 4 Comparison Between Sugarcane, Sugar Beet, and Sweet Sorghum on Agronomic Traits and Ethanol Production Parameters

Traits	Sugarcane	Sugar Beet	Sweet Sorghum
Crop duration	About 7 months	About 5-6 months	About 4 months
Growing season	Only one season	Only one season	Temperate: 1 season; Tropical: 2/3 seasons
Soil requirement	Grows well in drain soil	Grows well in sandy loam; also tolerates alkalinity	All types of drained soil
Water management ($m^3 h^{-1}$)	36,000	18,000	4000-8000
Crop management	Requires good management	Greater fertilizer requirement; requires moderate management	Little fertilizer required; less pest and disease complex; easy management
Yield per ha (tons)	70-80	30-40	54-69
Sugar content on weight basis (%)	10-12	15-18	7-12
Sugar yield (tons ha^{-1})	7-8	5-6	6-8
Ethanol production from juice ($L\,ha^{-1}$)	3000-5000	5000-6000	3000
Harvesting	Mechanically harvested	Very simple; normally manual	Very simple; both manual and mechanical harvest

Source: Almodares and Hadi (2009) and Rao et al. (2009).

7 MICROBE-ASSISTED PHYTOREMEDIATION

Since phytoremediation is a time-consuming process, it can be enhanced by a plant microbe-mediated approach, popularly referred as biophytoremediation, because many microorganisms have been reported for their HM tolerance and removal of a broad spectrum of metal species. Bacterial cells (approximately 1.0-1.5 mm^3) have an extremely higher surface area-to-volume ratio, which influences HM absorption, than inorganic soil components by a metabolism-independent, passive/metabolism-dependent active process (Ledin et al., 1996). The major microbial groups—bacteria, fungi, yeast, and algae—play a better role in bioremediation by their biosorption properties. Some of the tested species includes *Bacillus subtilis*, *Rhizopus arrhizus*, *Saccharomyces cerevisiae*, and *Scytonema hofmanni* (Vijayaraghavan and Yun, 2008). Though the microbial biosorbents are cheaper and more effective alternatives for HM removal, effectiveness can be attained mainly in aqueous solutions (Joshi and Juwarkar, 2009; Kapoor et al., 1999; Wang and Chen, 2009).

Plant growth-promoting (PGP) microbes, a group of microbes found in rhizosphere or in association with the roots or other plant parts as endophytes, can play a key role in this scenario (Glick et al., 1999). The higher bacterial biomass in the rhizosphere occurs because of the nutrient release (especially small molecules such as amino acids, sugars, and organic acids) from the roots. In turn the PGP bacteria will support for plants by various direct (nitrogen fixation, phosphate solubilization, iron chelation, and phytohormone production) or indirect (suppression of plant pathogenic organisms, induction of resistance in host plants against plant pathogens and abiotic stresses) mechanisms (Penrose and Glick, 2001).

A wide range of PGP microbes is able to alleviate HM stress in soil by enhancing the HM uptake of plants as well as by increasing plant growth. Some of them are *Rhizobium*, *Pseudomonas*, *Agrobacterium*, *Burkholderia*, *Azospirillum*, *Bacillus*, *Azotobacter*, *Serratia*, *Alcaligenes*, and *Arthrobacter* (Carlot et al., 2002; Glick, 2003). A detailed review of PGP bacteria and their role in phytoremediation has been provided by many researchers (Ma et al., 2011; Rajkumar et al., 2012) and also demonstrated experimentally on various agricultural crops such as *Zea mays*, *Vigna mungo*, and *H. annuus* (Ganesan, 2008; Jiang et al., 2008; Rajkumar et al., 2008) and hyperaccumulating crops such as *Alyssum murale* and *Salix caprea* (Abou-Shanab et al., 2008; Kuffner et al., 2008). The following mechanisms were suggested for HM removal by microbes.

Siderophores: The low-molecular mass (400-1000 Da) compounds with high-association constants for complexing Fe and also other metals Al, Cd, Cu, Ga, In, Pb, and Zn (Rajkumar et al., 2010). Production of siderophores by PGP *Pseudomonas* spp., its metal complexes formation with high solubility, and the resulting higher HM uptake by plant has been reported (Wu et al., 2006a,b).

Organic acids: Low-molecular-weight organic acids of PGP microbes bind to metal ions in soil solution and increase the metal bioavailability to plants. However, the stability of the ligand:metal complexes is dependent on several factors such as the nature of organic acids (number of carboxylic groups and their position), the binding form of the HMs, and pH of soil solution (Saravanan et al., 2007).

Biosurfactants: The amphiphilic molecules with nonpolar tail and polar/ionic head, produced by microbes, are able to form complexes with HMs at the soil interface, desorbing metals from soil matrix and thus increasing metal solubility/bioavailability in the soil solution. Several studies have demonstrated the role of microbial biosurfactants in facilitating the release of adsorbed HMs and in enhancing the phytoextraction potential of plants. Still, documentation under field conditions is lacking (Basak and Das, 2014; Franzetti et al., 2014).

Polymeric substances and glycoprotein: Extracellular polymeric substances, mucopolysaccharides, and proteins produced by plant-associated microbes can make complexes with HMs and decrease their mobility in soils. Glomalin, an insoluble glycoprotein produced by arbuscular mycorrhizal fungi, proved its ability to form complexes with HMs Cu, Pb, and Cd (Bano and Ashfaq, 2013; Foster et al., 2000; Liu et al., 2001).

Metal reduction and oxidization: Mobility of HMs can be enhanced through oxidation and reduction reactions of plant-associated Fe- and S- oxidizing bacteria. Use of Fe-reducing bacteria and the Fe/S oxidizing bacteria together showed significantly increased mobility of Cu, Cd, Hg, and Zn, which might be due to coupled and synergistic metabolism of oxidizing and reducing microbes (Beolchini et al., 2009; Shi et al., 2011; Wani et al., 2007).

Biosorption: The plant-associated microbes may contribute to plant metal uptake through biosorption (microbial adsorption of soluble/insoluble organic/inorganic metals). Among the microbes, mycorrhizal fungi are key partners. The large surface area, cell wall (chitin, extracellular slime, etc.) and intracellular compounds (metallothioneins, P-rich amorphic material) of fungi endow them with a strong capacity for HM absorption from soil (He and Chen, 2014; Volesky and Holan, 1995).

The other interesting mechanism by which PGP microbes can alleviate HM stress on plant growth is through the production of enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which controls the production of ethylene, a stress hormone (Glick, 2014). Similarly production of indole-3-acetic acid by rhizobacteria can enhance HM uptake by plant (Zaidi et al., 2006). The role of endophytic bacteria interacting with their host plant is of significance in the process of phytoremediation. Under

HM stress, the endophytes with the ability of stress tolerance can alleviate the stress and allocate the metals to the plant shoot (Ma et al., 2011; Weyens et al., 2009).

Few reports are available on the assistance of PGP microbes in HM remediation by sweet sorghum. The report of Duponnois et al. (2006) documents that the inoculation of Cd-tolerant *Pseudomonas* strains, mainly *P. monteilii*, showed significantly improved Cd uptake by sorghum plants under glasshouse conditions. Measurement of catabolic potentials on 16 substrates showed that pseudomonad strains presented a higher use of ketoglutaric and hydroxybutyric acids, as opposed to fumaric acid in control soil samples. It is suggested that fluorescent pseudomonads could act on the effect of small organic acids on phytoextraction of HMs from soil. Subsequently, Abou-Shanab et al. (2008) examined the ability of four bacterial isolates (*B. subtilis*, *Bacillus pumilus*, *Pseudomonas pseudoalcaligenes*, and *Brevibacterium halotolerans*) on HM removal capacity of sorghum. Sorghum roots accumulated higher concentration of Cr followed by metals Pb, Zn, and Cu. A comparative analysis was done on phytoremediation efficiency of sweet sorghum, *Phytolacca acinosa* and *Solanum nigrum*, with the inoculation of PGP endophyte *Bacillus* sp. SLS18 on Mn- and Cd-amended soils. Sweet sorghum was found to have higher metal absorption (Mn vs. Cd; 65% vs. 40%) than *P. acinosa* (Mn vs. Cd; 55% vs. 31%) and *S. nigrum* (Mn vs. Cd; 18% vs. 25%). The effect of this remediation process on biomass was also observed in the order of sweet sorghum > *P. acinosa* > *S. nigrum*.

8 WORK AT ICRISAT

ICRISAT has developed several improved hybrid parental lines of sweet sorghum with high stalk sugar content that are currently being tested in pilot studies for sweet sorghum-based ethanol production in India, the Philippines, Mali, and Mozambique. Concerted research efforts under National Agricultural Research System (NARS) have led to the development and release of cultivars like SSV 84, CSV 19SS, CSH 22SS, and CSV 24SS for all India cultivation with productivity ranging from 40 to 50 tons ha^{-1} (Vinutha et al., 2014). Trial data over 3 years (2005-2007) and six seasons indicated that there is no reduction in grain yield while improving the sugar yield. Sugar yield and associated traits have greater genotype × environment interaction; therefore, it is prudent to breed for season-specific hybrids.

ICRISAT launched a global **BioPower** initiative in 2007 to find ways to empower the dryland poor to benefit from emerging opportunities in renewable energies. This involves the collaborative partnership of NARS, particularly India, the Philippines, Mali, and private sector partners in Brazil, the United States, Germany, and Mexico. ICRISAT focuses on hybrids parent development to produce cultivars withstanding biotic and abiotic stresses thereby strengthening sweet sorghum value chains and their impact. The ICRISAT has made the first attempt in India to evaluate and identify useful high biomass producing sweet sorghum germplasm from world collections. The sweet sorghum program at ICRISAT mainly focuses on developing primarily hybrid parents adapted to rainy and post-rainy seasons due to the highly significant interaction of genotype by environment (G × E). However about 100 sweet sorghum varieties and restorer lines and 50 improved hybrids were identified. ICSV 93046, ICSV 25274, ICSV 25280, and ICSSH 58 were identified for release owing to their superior performance in All India Coordinated Sorghum Improvement Project (AICSIP) multilocation trials during 2008-2012 (Rao et al., 2013). Sweet sorghum improvement aims for simultaneous improvement of stalk sugar traits such as total soluble sugars or (brix %), green stalk yield, juice quantity, girth of the stalk, and grain yield. Conventional breeding approaches are practiced for an increase in sucrose yield; R lines showed a brix percentage of 12-24% in the rainy season and 9-19% in the post-rainy season. In total, 600 A/B pairs were screened at ICRISAT and the brix percentage ranged from 10% to 15% in the rainy season and 8% to 13% in the post-rainy season (Rao et al., 2009). Sweet sorghum bagasse is highly palatable and intake by livestock is more than normal sorghum stover (Blummel et al., 2009).

Some insect- and pest-resistant materials have been developed at ICRISAT, such as ICSR 93034 and ICSV 700. ICSV 93046 (ICSV 700 × ICSV 708) is a promising sweet sorghum variety tolerant to shoot fly, stem borer, and leaf diseases; it also displays stay-green stems and leaves even after physiological maturity and has good grain (3.4-4.1 tons ha^{-1}) and biomass yield. Another hybrid, ICSSH 72, shows excellent fodder quality in the rainy season and is resistant to leaf diseases. SPV 422 also exhibits resistance to leaf diseases and other hybrids developed at ICRISAT, India; for example, ICSSH 21 (ICSA 38 × NTJ 2) and ICSSH 58 (ICSA 731 × ICSV 93046) are under advance testing stages. ICSSH 30 variety shows superior grain yields in both rainy and post-rainy seasons whereas ICSSH 39 and 28 are best for sugar yield. ICSSH 24 variety is supposed to be best suited for the rainy season (Vinutha et al., 2014). Some of the varieties and hybrids developed from ICRISAT are given in Table 5.

9 WORK AT INDIAN NARS

Concerted research efforts at AICSIP centers have resulted in the identification of several promising sweet sorghum varieties such as SSV 96, GSSV 148, SR 350-3, SSV 74, HES 13, HES 4, SSV 119, and SSV 12611 for total soluble solids (TSS%) and juice yield during 1991-1992 trials, GSSV 148 for cane sugar during 1993-1994 trials, NSS 104 and HES 4 for green cane yield, juice yield, juice extraction, and total sugar content during 1999-2000 trials, and RSSV 48 for better alcohol yield during 2001-2002. An evaluation of 11 promising sweet sorghum varieties bred at different AICSIP centers indicated superiority of the varieties NSSV 255 and RSSV 56 for green cane yield, juice yield, juice extractability, commercial cane sugar (CCS) yield (q ha^{-1}), and percent nonreducing sugars over the rest of the varieties. The varieties RSSV 79, PKV809, NSSV 256, and NSSV 6 excelled the check with superior performance for green cane yield, juice yield, juice extractability, CCS yield, and total sugars (Reddy et al., 2007).

The Rusni distillery, established in 2007 near Sangareddy in the Medak district of Telangana, India, was the first sweet sorghum distillery amenable to use multiple feedstocks for transport-grade ethanol production. It generated 99.4% of fuel ethanol with a total capacity of 40 kiloliters per day (KLPD). It also produced 96% extra neutral alcohol (ENA) and 99.8% pharma alcohol from agro-based raw materials such as sweet sorghum juice, molded grains, broken rice, cassava, and rotten fruits. ICRISAT has incubated sweet sorghum ethanol production in partnership with Rusni Distilleries through its Agri-Business Incubator. A pilot-scale sweet sorghum distillery of 30 KLPD capacity was established in 2009 at Nanded, Maharashtra. It used commercially grown sweet sorghum cultivars such as CSH 22SS, ICSV 93046, sugargrace, JK Recova, and RSSV 9 in the 25 km radius of the distillery to produce transport-grade ethanol and ENA during 2009-2010. However, it could not continue operations due to the low mandated ethanol price. The Cabinet Committee of Economic Affairs (CCEA) of GOI on November 22, 2012, recommended 5% mandatory blending of ethanol with gasoline (Aradhey and Lagos, 2013). The government's current target of 5% blending of ethanol in gasoline has been partially successful in years of surplus sugar production and unfulfilled when sugar production declines.

Table 5 Sweet sorghum Varieties and Hybrids Developed from ICRISAT

Varieties				Hybrids		
ICSV 93046	ICSSV 10032	ICSSV 10059	ICSSV 12021	ICSSH 1	ICSSH 28	ICSSH 55
ICSSV 10001	ICSSV 10033	ICSSV 10060	ICSSV 12022	ICSSH 2	ICSSH 29	ICSSH 56
ICSSV 10005	ICSSV 10034	ICSSV 10061	ICSSV 12023	ICSSH 3	ICSSH 30	ICSSH 57
ICSSV 10006	ICSSV 10035	ICSSV 10062	ICSSV 12024	ICSSH 4	ICSSH 31	ICSSH 58
ICSSV 10007	ICSSV 10036	ICSSV 10063	ICSSV 12025	ICSSH 5	ICSSH 32	ICSSH 59
ICSSV 10008	ICSSV 10037	ICSSV 10064	ICSSV 12026	ICSSH 6	ICSSH 33	ICSSH 60
ICSSV 10009	ICSSV 10038	ICSSV 10065	ICSSV 12027	ICSSH 7	ICSSH 34	ICSSH 61
ICSSV 10010	ICSSV 10039	ICSSV 10066	ICSSV 14001	ICSSH 8	ICSSH 35	ICSSH 62
ICSSV 10011	ICSSV 10040	ICSSV 10067	ICSSV 14002	ICSSH 9	ICSSH 36	ICSSH 63
ICSSV 10012	ICSSV 10041	ICSSV 10068	ICSSV 14003	ICSSH 10	ICSSH 37	ICSSH 64
ICSSV 10013	ICSSV 10042	ICSSV 10069	ICSSV 14004	ICSSH 11	ICSSH 38	ICSSH 65
ICSSV 10014	ICSSV 10043	ICSSV 12005	ICSSV 14005	ICSSH 12	ICSSH 39	ICSSH 66
ICSSV 10015	ICSSV 10044	ICSSV 12006	ICSSV 12020	ICSSH 13	ICSSH 40	ICSSH 67
ICSSV 10016	ICSSV 10045	ICSSV 12007	ICSSV 12021	ICSSH 14	ICSSH 41	ICSSH 68
ICSSV 10017	ICSSV 10046	ICSSV 12008	ICSSV 12022	ICSSH 15	ICSSH 42	ICSSH 69
ICSSV 10018	ICSSV 10047	ICSSV 12009	ICSSV 12023	ICSSH 16	ICSSH 43	ICSSH 70
ICSSV 10019	ICSSV 10048	ICSSV 12010	ICSSV 12024	ICSSH 17	ICSSH 44	ICSSH 71
ICSSV 10021	ICSSV 10049	ICSSV 12011	ICSSV 12025	ICSSH 18	ICSSH 45	ICSSH 72
ICSSV 10022	ICSSV 10050	ICSSV 12012	ICSSV 12026	ICSSH 19	ICSSH 46	ICSSH 73
ICSSV 10024	ICSSV 10051	ICSSV 12013	ICSSV 12027	ICSSH 20	ICSSH 47	ICSSH 74
ICSSV 10025	ICSSV 10052	ICSSV 12014	ICSSV 14001	ICSSH 21	ICSSH 48	ICSSH 75
ICSSV 10026	ICSSV 10053	ICSSV 12015	ICSSV 14002	ICSSH 22	ICSSH 49	ICSSH 76
ICSSV 10027	ICSSV 10054	ICSSV 12016	ICSSV 14003	ICSSH 23	ICSSH 50	ICSSH 77
ICSSV 10028	ICSSV 10055	ICSSV 12017	ICSSV 14004	ICSSH 24	ICSSH 51	ICSSH 78
ICSSV 10029	ICSSV 10056	ICSSV 12018	ICSSV 14005	ICSSH 25	ICSSH 52	
ICSSV 10030	ICSSV 10057	ICSSV 12019		ICSSH 26	ICSSH 53	
ICSSV 10031	ICSSV 10058	ICSSV 12020		ICSSH 27	ICSSH 54	

The interim price of US \$0.44 l^{-1} would no longer hold as the price would now be decided by market forces. It is expected this decision will have a positive effect on forthcoming distilleries in India.

10 WORK IN OTHER COUNTRIES

Other countries involved in sweet sorghum research and development are the United States, Brazil, Colombia, Haiti, Argentina, Italy, Germany, Hungary, France, China, the Philippines, and Indonesia. Nonfood crops and materials such as cassava and sweet sorghum are the priority choice for biofuel ethanol production in China. Sorghum Research Institute (SRI) of Liaoning Academy of Agricultural Sciences (LAAS) is the lead organization involved in sweet sorghum research in China since the 1980s. So far 17 promising sweet sorghum hybrids were released nationally. A few industries such as ZTE energy company limited

(ZTE, Inner Mongolia), Fuxin Green BioEnergy Corporation (FGBE), Xinjiang Santai Distillery, Liaoning Guofu Bioenergy Development Company Limited, Binzhou Guanghua Biology Energy Company Ltd, Jiangxi Qishengyuan Agri-Biology Science and Technology Company Ltd, Jilin Fuel Alcohol Company Limited, and Heilongjiang Huachuan Siyi Bio-fuel Ethanol Company Ltd conducted either large-scale sweet sorghum processing trials or are at the commercialization stage (Reddy et al., 2011).

In the Philippines, sweet sorghum has been proven to be a technically and economically viable alternative feedstock for bioethanol production. The plantation, agronomic performance, and actual bioethanol production of sweet sorghum have been evaluated on different plantation sites nationwide. A hectare of sweet sorghum plantation can potentially provide farmers with an annual net income of US $1860.47 at a stalk-selling price of US $22 and grain price of US $0.30. The San Carlos Bioenergy Inc. (SCBI) became the first commercial distillery to process sweet sorghum bioethanol in Southeast Asia under the Department of Agriculture (DA) and produced 14,0001 of fuel-grade ethanol in 2012 (Demafelis et al., 2013). The Ecofuels 300 KLPD distillery at San Mariano, Isabela, is planning to use sugarcane and sweet sorghum as feedstocks for ethanol production commercially. The sweet sorghum growers are enthusiastic as the ratoon (new shoot) yields are about 20-25% higher than that of plant crop. The Bapamin enterprises based in Batac have been successfully marketing vinegar and hand sanitizer made from sweet sorghum since 2009 (Reddy et al., 2011).

In the United States, a sweet sorghum distillery is under construction in South Florida by South Eastern Biofuels Ltd. In Brazil, large-scale sweet sorghum pilot trials are being conducted in the last 3 years by Ceres Inc, Chromatin Inc, Advanta Inc, Dow Agro Sciences, as well as Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) in the areas of sugarcane renovation to commercialize sweet sorghum (Nass et al., 2007). The Government of Brazil has identified 1.8 m ha for sweet sorghum plantation to augment fuel-grade ethanol production. In some African countries like Mozambique, Kenya, South Africa, and Ethiopia, sweet sorghum adaptation trials are being conducted in pilot scale to assess feasibility of sweet sorghum for biofuel production.

11 FUTURE OUTLOOK

The phytoremediation potential of sweet sorghum has not been studied extensively enough under field conditions. The published data has come from trials using pot conditions or microcosm experiments, which are not adequate for future cleanup of contaminated areas. In an economic viability assessment done by Basavaraj et al. (2013) on ethanol production from sweet sorghum, net present value (NPV), the indicator of economic viability assessment, is found to be negative. So, it may be difficult for the industry to take off under the current scenario of fluctuating ethanol price, feedstock price, and ethanol recovery rate. A well-developed technology for phytoremediation and ethanol production involving sweet sorghum along with well-defined policy support is crucial to meet future blending requirements and also to improve rural while adhering to greenfield biorefinery approach.

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