Nutrient composition of spent wash and its impact on sugarcane growth and biochemical attributes

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Published online: 6 October 2011

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Abstract Nutrient composition of crude and digested spent wash and effect of their application on sugarcane growth and biochemical attributes were studied. Higher concentrations of essential nutrients (P, S, Fe, Mn, Zn, Cu) and heavy metals (Cd, Cr, Ni and Pb) were present in crude spent wash (CSW) as compared to the digested spent wash (DSW); sulphur content was the highest ($765 \mu\text{g ml}^{-1}$ in DSW and $1,609 \mu\text{g ml}^{-1}$ in CSW) among all nutrients analyzed. Sugarcane (*Saccharum* spp. hybrid cultivar CoLk 8102) setts grown in soil pot culture conditions with different rates of crude spent wash (5, 10, 20 and 100 ml kg^{-1} soil) along with digested spent wash (100 ml kg^{-1} soil) showed improvement in bud sprouting (10.5 %), settling height (40 %), root number (9.4 %), root length (13.2 %), chlorophyll a (52.9 %) and b (55.3 %) contents and activity of catalase (98 %) enzyme over control at low rate of crude spent wash (5 ml kg^{-1} soil). Whereas, higher doses of spent wash (20 and 100 ml kg^{-1} soil) decreased these parameters markedly except peroxidase which was found higher at all the levels of both CSW and DSW. Findings indicated stimulatory effect of low rate of crude spent wash (5 ml kg^{-1} soil) on root and shoot growth and inhibitory effect of higher dose (100 ml kg^{-1} soil) of both crude and digested spent wash, therefore, judicious application of spent wash will improve crop productivity and alleviate environmental pollution problems.

Keywords Spent wash · Nutrients · Growth attributes · Chlorophyll · Enzyme activity

Introduction

Distillery effluent or spent wash (SW) is usually considered as waste of distillery processes and can be classified as a dilute organic liquid fertilizer with high potassium content (Samuels 1980). In recent years, due to expansion of distilleries in sugar cane growing countries, the disposal of SW has become an acute problem. In India, about 15,000 million liters of spent wash is produced annually from 246 distilleries which is characterized by undesirable color, foul odor, high biological oxygen demand (BOD: $5,000\text{--}8,000 \text{ mg l}^{-1}$) and chemical oxygen demand (COD: $25,000\text{--}30,000 \text{ mg l}^{-1}$) (Joshi et al. 1994). Such situation has created an acute problem of spent wash disposal with the expansion of distilleries in the sugarcane growing countries. Spent wash whether treated or not, is used to irrigate sugarcane as well as to provide nutrients to crop as it also contains the essential plant nutrients required for its growth (Samuels 1980; Yadav et al. 2010). Besides, it also contains heavy metals (Srivastava and Jain 2010). As a result, it may exert deleterious effect on sugarcane growth if the heavy metals are present in amount exceeding their toxicity limits due to higher application of spent wash. Spent wash also contaminates drinking water for human beings and animals and irrigation water. Industrial effluents containing heavy metals also pollute the cultivated fields and underground water either through seepage or letting these into the fields nearby. The total amount of Pb may range from 2 to 200 ppm in such agricultural lands. The judicious use of SW will help to improve soil health and crop productivity and alleviate environmental pollution problems (Deverajan and Oblisami 1995). Its application to soil at low concentration has been reported to be beneficial to increase sugar cane (Mohamed Haron and Subash Chandra Bose 2004), rice (Deverajan and Oblisami

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1995), wheat and rice (Pathak et al. 1998), mustard yield (Malaviya and Sharma 2011) and ground nut quality and physiological response of soybean (Ramana et al. 2001). Diluted spent wash could be used for irrigation purpose without adversely affecting soil fertility (Raverkar et al. 2000; Kaushik 2005) and crop productivity (Ramana et al. 2001). Spent wash irrigation (33 %) increased the yield of pulses (Chidankumar et al. 2010). In present investigation, an attempt has been made to determine the nutrient composition of crude and digested spent wash and their impact on germination, growth and biochemical attributes of sugarcane.

Materials and methods

Single bud setts of sugarcane (*Saccharum* spp. hybrid) cultivar CoLk 8102 were grown in pot culture conditions containing farm soil (pH 6–7) with different rates of applied crude spent wash (BOD 40,000 mg l⁻¹, pH 4.0) (5, 10, 20 and 100 ml kg⁻¹ soil) along with a digested spent wash (BOD: 4,000 mg l⁻¹, pH 9.0) (100 ml kg⁻¹ soil) and control (water treatment). The pots were kept under net house conditions (day temperature 31.1–37.6 °C; night temperature 15.6–19.7 °C). Data on bud sprouting and growth attributes were recorded for 30 days after planting. Chlorophyll contents were determined in fresh leaves by the method as described earlier (Jain and Shrivastava 2003).

For enzyme assay, fresh leaf samples were ground and extracted (2 ml 0.2 g FW) in 50 mM sodium phosphate buffer, pH 7.0 containing 2 mM EDTA and 5 mM β-ME. The homogenate was centrifuged at 10,000 g for 10 min at 4 °C. The supernatant was dispensed into aliquots for further analyses.

Catalase (CAT) was assayed by the methods described earlier (Jain and Shrivastava 2003). For peroxidase assay, the reaction mixture containing 5 ml 0.1M phosphate buffer, pH 6.0, 1 ml 0.01 % H₂O₂, 1 ml 0.5 % *p*-phenylenediamine and enzyme extract (0.1 ml) was incubated for 5 min at 25 °C (Luck 1963). The color developed was measured at 485 nm, and the peroxidase activity was expressed as units mg⁻¹ protein. Soluble protein was determined in enzyme extracts according to Lowry et al. (1951).

Nutrient and heavy metal contents were determined in both crude spent wash (CSW) and digested spent wash (DSW) after wet digestion in a di- acid mixture (HNO₃: HClO₄) in 10: 1 ratio as described by Jain et al. (2000). Nutrient contents viz., phosphorus (P) (Jain et al. 2011), sulphur (S) (Beaton et al. 1968), micronutrients (Fe, Mn, Zn, Cu) and heavy metals (Cd, Cr Ni and Pb) were determined in clear digests by atomic absorption spectrophotometer (AAS) and expressed as µg ml⁻¹ spent wash. All the data presented are the mean of three replications.

Statistical analysis

Standard deviation (±SD) was calculated using means of three replicates as described by Panse and Sukhatme (1985).

Results and discussion

Spent wash is the residual liquid waste generated during alcohol production and pollution caused by it is one of the most critical environmental issues. Despite standards imposed on effluent quality, untreated or partially treated effluent very often finds access to water courses. The distillery wastewater with its characteristic unpleasant odor poses a serious threat to the water quality in several regions around the globe. The ever increasing generation of distillery spent wash on the one hand and stringent legislative regulations of its disposal on the other has stimulated the need for developing new technologies to process this effluent efficiently and economically including plant growth and yield (Sarayu et al. 2009).

In present study, analysis of crude and digested spent wash indicated very high concentrations of P, S, Fe, Mn, Zn and Cu and a number of heavy metals in CSW as compared to those in DSW. Concentrations (µg ml⁻¹) in both DSW and CSW, respectively were 35.1, 77.6 (P), 765, 1609 (S), 9.52, 68.5 (Fe), 0.72, 3.0 (Mn), 0.72, 4.01 (Zn) 0.38, 2.66 (Cu), 0.004, 0.025 (Cd), 0.95, 0.172 (Cr), 0.88, 0.86 (Ni) and 0.54, 1.24 (Pb). Lead (Pb) in CSW had the highest concentration among all heavy metals analyzed (Table 1).

Very low rates of application of CSW (5 ml kg⁻¹ soil) showed improvement in bud sprouting (10.5 %), settling height (40 %), root number (9.4 %), root length (13.2 %) (Fig. 1). Whereas, high rates of CSW (20, 100 ml kg⁻¹ soil) as well as DSW (100 ml kg⁻¹ soil) decreased these

Table 1 Essential nutrients and heavy metal contents (µg ml⁻¹) in spent wash

Metals (µg ml ⁻¹)	Digested spent wash (pH, 9.0)	Crude spent wash (pH, 4.0)
P	35.1±2.76	77.6±2.51
S	765±53.5	1,609±37.3
Fe	9.52±1.43	68.5±3.04
Mn	0.72±0.03	3.0±0.17
Zn	0.72±0.04	4.01±0.52
Cu	0.38±0.03	2.66±0.14
Cd	0.004±0.001	0.025±0.01
Cr	0.950±0.04	0.172±0.01
Ni	0.880±0.04	0.863±0.05
Pb	0.54±0.05	1.24±0.12

Values are mean of three replicates ± SD

Fig. 1 Effect of spent wash on bud sprouting (a), settling height (b), root number (c) and root length (d) of sugarcane. Vertical bar represents $\pm$ SD of mean of three replicates. CSW, Crude spent wash; DSW, Digested spent wash

attributes; decrease was about 17.4–50 % in bud sprouting, 6.7–33.3 % in settling height, 31.2–68.8 % in root number, and 36.2–64.6 % in root length; highest decrease was observed at 100 ml CSW kg⁻¹ soil (Fig. 1). Adverse effects of crude spent wash were higher as compared to the digested one. Similar to sugarcane, lower concentration of distillery effluent (10 %) was found favorable for seed germination in forest seedbeds (Pandey and Soni 1994) and vegetables (Chandrabu and Basavaraju 2007). Improvement in growth and bud germination due to diluted SW were also obtained by Nallathambhi et al. (1999). Mahimairaja and Bolan (2004) also found that low doses of distillery effluent remarkably improve growth and yield of dry land crops (ragi, groundnut, sorghum and green gram). Experiments conducted in the major sugarcane growing areas in Guanxi, China showed inhibitory effects of vinasse (liquid waste or spent wash) on seed cane emergence (Li et al. 2007). The distillery effluent exhibited the significant deleterious effect

on seed germination and early seedling growth in pigeon pea (Karande and Ghanvat 1994). Most seed cane buds could not sprout probably due to the anaerobic conditions produced by application of vinasse due to its high BOD and COD. This may be attributed to the nutritional imbalance as well as reduction in the availability of essential nutrients to the plants due to increase in the osmotic pressure of soil irrigated with higher concentration of effluent (Ramana et al. 2001). Diluted spent wash increases the growth of shoot length, leaf number, leaf area and chlorophyll contents (Rani and Srivastava 1990).

Plants supplied with graded concentrations of CSW exhibited variations in chlorophyll a and b contents. Very low rates of CSW (5 ml kg⁻¹ soil) showed the highest chlorophyll a (2.4 mg g⁻¹ fwt) and b (0.73 mg g⁻¹ fwt) contents, while minimum contents of chlorophyll a (0.33 mg g⁻¹ fwt) and chlorophyll b (0.11 mg g⁻¹ fwt) were observed at 100 ml kg⁻¹ soil CSW (Fig. 2). The

Fig. 2 Effect of spent wash on chlorophyll content (a), activity of catalase (b), peroxidase (c) and soluble protein content (d) of sugarcane. Vertical bar represents $\pm$ SD of mean of three replicates. CSW, Crude spent wash; DSW, Digested spent wash

increase in chlorophyll content in low concentration spent wash treatment suggests that synthesis of chlorophyll is accelerated in low concentration. Similar findings have been reported by many workers (Rani and Srivastava 1990; Orhue et al. 2005; Rath et al. 2011). Some of the possible reasons for the decrease of pigment contents may be due lower availability of essential nutrients due to poor root growth (Rath et al. 2011).

The high rate of CSW (100 ml kg⁻¹ soil) exhibited minimum activity of catalase enzyme (31.6 µmol H₂O₂ decomposed mg⁻¹ protein), while, maximum activity was obtained at the low rate of CSW (5 ml kg⁻¹ soil) (97 µmol H₂O₂ decomposed mg⁻¹ protein) (Fig. 2). In contrast to this, Baghel (2008) found increased activity of catalase enzyme pea plant with an increase in concentration of treated distillery effluent indicating toxic and inhibitory effect of distillery effluent. The specific activity of peroxidase enzyme showed improvement at all the levels of spent wash indicating general response of variable nutrient stress. Increased activity of peroxidase in response to spent wash was observed by other workers also (Singh et al. 2003; Baghel 2008). Lower content of soluble protein was observed in leaf tissues of treated plants; untreated control having maximum content (Fig. 2).

These findings indicated stimulatory effects of very low rates of CSW (5 ml kg⁻¹ soil) application on sugar cane growth attributes, chlorophyll content and enzyme activity. However, high rates of CSW (100 ml kg⁻¹ soil) exhibited significant inhibitory effects on these attributes which may be due to higher availability of toxic metals (Cd and Pb) in growing medium and thus toxic to sugarcane plants. Inhibitory effect of higher doses (100 ml kg⁻¹ soil) of spent wash on root and shoot growth of sugarcane led to alteration in mineral composition, metabolic activities and mitotic activity (Srivastava and Jain 2010) which in turn resulted in reduction in sugarcane growth. Therefore, judicious use of spent wash will help to improve soil health and crop productivity and alleviate environmental pollution problems.

References

- Baghel RS (2008) Toxicity of distillery effluent seed germination, seedling growth and metabolism in *Pisum sativum*. Res Environ Life Sci 1:29–32
- Beaton JD, Burns GR, Platou S (1968) Determination of sulphur in soils and plant material. Technical Bul 14. The Sulphur Institute, Wash, DC
- Chandrasekhar S, Basavaraju HC (2007) Impact of distillery spent wash on seed germination and growth of leaves vegetables: an investigation. Sugar J (SISSTA) 38:20–50
- Chidankumar CS, Chandrasekhar S, Girijanagendraswamy NR (2010) Nutritional additives of spent wash on pulses production. J Biopestic 3:51–54
- Deverajan L, Oblisami G (1995) Effect of distillery effluent on soil fertility status, yield and quality of rice. Madras Agric J 82:664–665
- Jain R, Shrivastava AK (2003) Biochemical studies on healthy and chlorotic sugarcane leaves. Sugarcane Int 1:3–5
- Jain R, Srivastava S, Madan VK (2000) Influence of chromium on growth and cell division of sugarcane. Indian J Plant Physiol 3:228–231
- Jain R, Solomon S, Chandra A (2011) Some discernible physio-biochemical changes associated with improved sprouting of sugarcane setts treated with ethephon. Sugar Tech 13:123–128
- Joshi HC, Karla N, Chaudhary A, Deb DL (1994) Environmental issues related with distillery effluent utilization in agriculture in India. Asia Pac J Environ Dev 1:92–103
- Karande SM, Ghanvat NA (1994) Effect of untreated effluents of Pravara pulp and paper mill and distiller on seed germination and early seedling growth in pigeon pea. Proc Acad Environ Biol 3:165–169
- Kaushik A (2005) Impact of long and short term irrigation of a sodic soil with distillery effluent in combination with bioamendments. Bioresour Technol 96:1860–1866
- Li YR, Zhu QZ, Wang WZ, Solomon S (2007) Pre-emergence application of vinasse on sugarcane growth and sugar productivity in China. Sugar Tech 9:160–165
- Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin Phenol reagent. J Biol Chem 193:65–275
- Luck H (1963) Peroxidase. In: Bergmeyer HU (ed) Methods in enzymatic analysis. Academic, New York, pp 895–897
- Mahimairaja S, Bolan NS (2004) Problems and prospects of agricultural use of distillery spentwash in India. In: Third Australian and New Zealand Soil Science Societies Joint Conference, Sydney, Australia, 5–9, December 2004
- Malaviya P, Sharma A (2011) Effect of distillery effluent on yield attributes of *Brassica napus* L. J Environ Biol 32:385–389
- Mohamed Haron AR, Subash Chandra Bose M (2004) Use of distillery spent wash for alkali soil reclamation, treated distillery effluent for ferti irrigation of crops. Indian Farming 3:48–51
- Nallathambhi P, Viswanathan R, Padmanaban P, Moharaj D, Jothi R (1999) Indian Sugar 49:111–123
- Orhue ER, Osaigbova AU, Vwioko DE (2005) Growth of maize (*Zea mays* L.) and changes in some chemical properties of an Utisol amended with brewery effluent. Afr J Biotechnol 4:973–978
- Pandey D, Soni P (1994) Distillery effluent—a potential resource for irrigating forest seed beds. Ambio 23:267–268
- Panse VG, Sukhatme P (1985) Statistical methods for agricultural workers, 4th edn. ICAR, New Delhi
- Pathak H, Joshi HC, Chaudhary A, Karla N, Dwivedi MK (1998) Distillery effluent as soil amendment for wheat and rice. J Indian Soil Sci 46:155–157
- Ramana S, Biswas AK, Kundu S, Saha JK, Yadava BR (2001) Effect of distillery effluent on seed germination in some vegetable crops. Bioresour Technol 82:273–275
- Rani R, Srivastava MM (1990) Eco-physiological response of *Pisum sativum* and *Citrus maxima* to distillery effluents. Int J Econ Environ Sci 16–23
- Rath P, Pradhan G, Misra MK (2011) Effect of distillery spent wash (DSW) and fertilizer on growth and chlorophyll content of sugarcane (*Saccharum officinarum* L.) plant. Recent Res Sci Technol 3:169–176
- Raverkar KP, Ramana S, Singh AB, Biswas AK, Kundu S (2000) Impact of post response of soyabean (*Glycine max* L.) to foliar application of distillery effluent. Ann Plant Soil Res 2:1–6
- Samuels G (1980) Rum distillery wastes: potential agricultural and industrial uses in Puerto Rico. P R Sugar J 43:9–12

- Sarayu MB, Acharya K, Madamwar D (2009) Distillery spent wash: treatment technologies and potential applications. *J Hazard Mater* 163:12–25
- Singh AB, Biswas AK, Ramana S (2003) Effect of distillery effluents on plant and soil enzymatic activities and groundnut quality. *J Plant Nutr Soil Sci* 166:345–347
- Srivastava S, Jain R (2010) Effect of distillery spent wash on cytomorphological behaviour of sugarcane seedlings. *J Environ Biol* 31:809–812
- Yadav DV, Jain R, Rai RK (2010) Impact of heavy metals on sugar cane. In: Sherameti I, Varma A (eds) *Soil heavy metals: soil biology series*, vol 19. Springer, Germany, pp 339–367