RESEARCH PAPER:

Microbial analysis of medicinal waste collected from industrial region of Punjab

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SUMMARY

Now a day's many industries have developed in Punjab state *viz.,* pharmaceutical, textiles, toy making, colouring, leather, tanning, electro-plating, paint and pigment manufacturing, metal plating etc. Industrial effluents are materials generally discarded from industrial operations or derived from manufacturing processes. A lot of effluents come from the pharmaceutical industries and cause water pollution. There is lot of waste materials which is discharged everyday from the large factories and mixed in the river waters causing water pollution. The sample collection was performed according to standard method. Physicochemical parameters like pH, temperature, turbidity, BOD, COD, DO were measured by using standard method. The microbial isolation was done by streak plate method on nutrient agar and on selective media for their identification. The final identification of recovered isolates was done by their biochemical testing accordance to the Bergey's Manual. Strains of *Staphylococcus aureus* isolated from sample I and strain of *Bacillus subtilis* isolated from sample II were identified. The resulted bacterial isolates, *Staphylococcus aureus* and *Bacillus subtilis* were highly pathogenic. *Staphylococcus aureus* is the most common cause of staph infections. The nature of *S. aureus* infections varies from minor cutaneous soft tissue infections to life-threatening endovascular infections such as endocarditis. *Bacillus subtilis* can contaminate food causing food poisoning. The bulk waste carrying the pathogenic bacteria may harm to the society.

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Improper disposal of pharmaceutical waste cause the adverse effects on environment and human health worldwide. Studies have demonstrated these adverse effects and have raised concerns about serious consequences that may occur if appropriate actions are not taken. A small desk survey was conducted in the spring of 2004 to get an overview on the development of waste research (Lagerkvist, 2006). The survey targeted the last 10 years of waste research at Swedish academic institutions trying to identify the total amount of research and trends over time with regard to issues, volume and distribution over academic disciplines. The results of the survey indicate that the academic waste research is very small in comparison to the R&D performed by the industry; there seems to be a lack of interaction between industry and academia and waste research is slowly getting into established academic. Pharmaceutical companies are progressively adopting and introducing the principles of quality by design with the main purpose of assurance and built in quality throughout the whole manufacturing process (Peinado *et al.*, 2010).

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Solid waste and its types:

Solid wastes are all the waste arising from human and animal activities that are normally solid and that are discarded as useless or unwanted. Solid waste includes organic waste (leaves, animal manure and agricultural waste) and problematic waste *e.g.* domestic and industrial waste, sewage sludge and municipal solid waste (Kumar, 2011).

Solid waste can be classified into different types depending on their sources: Household waste is generally classified as municipalwaste, Industrial waste as hazardous waste, and biomedical waste or hospital waste as infectious waste.

Municipal solid waste consists of household waste, construction and demolition debris, sanitation residue, and waste from streets. This garbage is generated mainly from

residential and commercial complexes. Over the last few years, the consumer market has grown rapidly leading to products being packed in cans, aluminum foils, plastics, and other such non-biodegradable items that cause incalculable harm to the environment. Industrial waste is the waste materials resulting from manufacturing, industrial and research and development processes and operations and which are not hazardous in accordance with the standards. Also included are nonhazardous oil spill cleanup waste, dry nonhazardous pesticides, dry nonhazardous chemical waste, and residue from the operations of a scrap metal shredding facility. Hospital waste is generated during the diagnosis, treatment, or immunization of human beings or animals or in research activities in these fields or in the production or testing of biological. It may include wastes like sharps, soiled waste, disposables, anatomical waste, cultures, discarded medicines, chemical wastes etc.

Consequences of microbes:

A huge quantity of pollutants in the form of domestic and industrial effluents is discharged directly or indirectly into the water bodies, which has severe impacts on its biotic and abiotic environment. The inorganic minerals like sodium, potassium, calcium, magnesium and heavy metals like iron, manganese, lead, mercury, chromium, cadmium, nickel, cobalt, beryllium copper etc. The pathogenic organisms of these wastes transmit to the water and pose serious problems. The analysis of river sediment is a useful method of studying environmental pollution with heavy metals (Batley 1989).

MATERIALS AND METHODS

Sample collection:

The samples were collected in the month of September 2010. Sampling site was selected to collect two medicinal wastes. The samples were collected in polythene bag of capacity 1 kg. The sample collection was performed according to standard method given by APHA, 1998. The solid wastes were carried to laboratory in well packed box sealed in ice to avoid the contamination and stored at 4 degree celsius (APHA, 1998).

Physicochemical parameters:

The colour of sample was determined by observing the sample very carefully and compared with standard solution. The odor of sample was determined by smelling and compared with standard solutions. Temperature, pH and turbidity were measured by thermometer, digital pH meter (pHep ®) and UV-VIS Spectrophotometer. BOD and COD were measured by titration method. For BOD

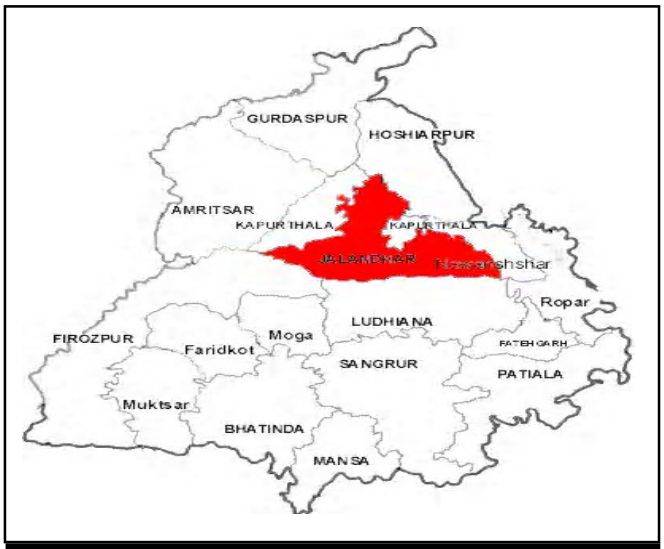

Fig. 1: Collection site Jalandhar (Punjab Region)

5 days incubation at 20^0 C and titration of initial and final DO (Li and Qiao *et al.*, 2004). For COD was determined by Dichromate oxidation and titration with ferrous ammonium sulphate.

Isolation of microorganisms:

Isolation of microorganism from solid waste (medicinal waste) was done by streaking them directly on Nutrient agar plates. The resulting microbial colonies obtained were further streaked on Nutrient agar plates to obtain individual isolated pure colony from each sample. The isolated pure colonies from each sample were preserved on Nutrient agar slants at -20^0C into the deep freezer.

Morphological characterization:

Colony size: Small, medium, large.

Margin: Circular, filamentous, rhizoid, irregular, entire, undulate lobate, curved.

Colour: White, pink, yellow, brown, green, metallic green.

Arrangement: Flat, raised convex, pulvinate, umbonate.

Texture: Smooth, flistering, rough, wrinkled, dry powdered.

Morphological characterization was also done by gram staining, negative staining and acid fast staining.

Biochemical characterization:

Biochemical characterization was done by imvic test (indole test, voges proskauer test, methyl red test and citrate utilization test), catalase test, sugar fermentation test. After biochemical test, two bacteria were isolated

from medicinal waste samples. Strains of *Staphylococcus* isolated from sample I and strain of *Bacillus* sp. isolated from sample II.

RESULTS AND DISCUSSION

Physicochemical parameters viz. colour, temperature, turbidity, odour, pH, DO, BOD and COD are listed in Table 1. Temperature of distilled water was 26.6^0 C. The temperature of two samples was 40^0 C and turbidity of distilled water was 4.98 NTU. Both the samples were turbid with fruity odour. OD of the sample I was 7.2 and sample II was 9.3. BOD of the sample I was 10.0 and sample II was 11.2. COD of the sample I was 15.2 and sample II was 18.5. pH of the sample I was 4 and sample II was 4.2 where as DO of the ddw was 6.28, pH was 6.91, BOD was 1.2 and COD was 1.

Table 1 : Physicochemical parameters

Parameter/s	Sample 1	Sample2
Colour	Dark brown	Dark brown
Temperature	40^oc	40^oc
Turbidity	turbid	turbid
Odour	fruity	fruity
pH	4.0	4.2
OD	7.2	9.3
BOD	10.0	11.2
COD	15.2	18.5

In microbial analysis, there was small colony of spherical cocci shape and grape like cluster arrangement found in isolate I but in case of isolate II, there was large rod shape colony and peritrichous arrangement as shown in Table 2.

Table 2 : Morphological characterizations

Microbial analysis	Isolate 1	Isolate 2
Colony	Small	Large
Shape	Spherical cocci	Rod
Arrangement	Grape like cluster	Peritrichous
Gram staining	Positive, grape like cluster	Positive, rod shape
Negative staining	Cocci shape	Rod shape
Acid fast staining	Grape like cluster	Straight rod

Strains of *Staphylococcus* sp. isolated from sample I and strain of *Bacillus* sp. isolated from sample II by biochemical characterization.

Staphylococcus aureus is the most common cause of staph infections. It is a spherical bacterium, frequently found in the nose and skin of a person. About 20% of the populations are long-term carriers of *S. aureus* (Belkum *et al.*, 1997). *S. aureus* was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses (Ogston, 1984). Each year some 500,000 patients in American hospitals contract a Staphylococcal infection (Bowersox, 1999). *Bacillus subtilis* is the well-studied gram positive bacterium. It is a useful model organism for the study of gene regulation, cell division, quorum sensing, and cellular differentiation. Its 4.2-Mb genome was one of the first genome completely sequenced. Genome sequencing reveals a number of interesting elements. The medicinal effluents contained two bacteria. Strains of *Staphylococcus* was isolated from sample I and strain of *Bacillus* sp. isolated from sample II. Both these organisms are potential pathogens of man capable of causing a variety of diseases. *Staphylococcus aureus* causes infections of the skin, deeper tissues and organs, pneumonia, enteritis and *Pseudomembraneous enterocolitis*, food poisoning. *Bacillus* cause is one of the largest pathogenic bacteria. *Bacillus subtilis*, the type species for the genus, is the most well studied gram positive bacterium. It is a useful model organism for the study of gene regulation, cell division, quorum sensing and cellular differentiation.

Conclusion:

Staphylococci are gram positive cocci that occur in grape- like clusters. They are ubiquitous and are the most common cause of localized suppurative lesions in human being. Their ability to develop resistance to penicillin and other antibiotics enhances their importance as a human pathogen, especially in hospital environment. *Bacillus* the rod shape bacteria are classified into two genera, the aerobic *Bacilli subtilis* and anaerobic *Clostridia*. The *Bacillus subtilis* consists of aerobic bacilli forming heat resistance spores. They are gram positive but tend to be decolorized easily so as to appear gram variable, or even frankly gram negative. They are generally motile with

Table 3 : Biochemical characterization

Sr. No.	Indole	MR	VP	Citrate utilization	Litmus milk reaction	Sugar fermentation Sucrose	Lactose	Dextrose	Catalase	Isolates
1.	-	+	+	-	Acid	+	-	+	+	*Staphylococcus aureus*
2.	-	-	+	-	Peptonization	+	-	+	+	*Bacillus subtilis*

peritrichous flagella. Members of this group exhibit great diversity in their property.

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