



RESEARCH PAPER

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Population load of targeted parasites and bacteria in pit latrine at Oshkhandas Valley, Gilgit-Baltistan, Pakistan

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Abstract

This study is conducted in 2012-2013 in Oshkhandas valley to assess the harmful implications of open defecation. The people of the area are deprived from the facility of modern toilet system. So they have constructed a vernacular system known as pit latrine (called as dasichukan in local language). This area has not been conferred a good sanitary system by the concerned departments. Hence this area is the good heaven for pathogens which are causing damage to the economy and health of the inhabitants of the area. Our research has shown that, annually a considerable number of infants, young ones, adults and old age people fall prey to the diseases by targeted bacteria (*E. coli*, *Salmonella*, *Shigella*) and parasites (*Ascaris lumbricoid*, *Trichuris trichuria*, *Gardialamblia* and *Cryptosporidium*). Neither vernacular nor scientific technique has been educated to the people of area to minimize the detrimental and injurious influence of the above said pathogens. The people were also using pit content as organic fertilizer without knowing the fact about how to properly compost it. They were handling with un-composted pit content without taking the available precautionary measures. In this connection this research was carried out and samples were taken from both pit latrine and agriculture field to assess the microbial load in pit and agriculture soil in different months. Our finding revealed that the population burden in pit versus soil did not differ significantly. So proper composting is inevitable to lessen the threats posed by the targeted bacteria and parasites. Overall 100 samples were taken 50 each from pit and 50 each from soil. The microbial population load in pit was observed as 3739/50g while the microbial burden in soil was recorded as 3226/50g and total bacterial and parasite load in both pit and soil was 6965.50/100g.

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Introduction

The annual amount of human excreta of one person corresponds to the amount of fertilizer needed to produce 250 kg of cereal which is also the amount of cereal that one person needs to consume per year (Heinonen-Tanska & van Wijk-Sijbesma, 2005).

Scientists have investigated that human urine is an important basis of nutrients that is used to varying extents for crop fertilization in countries such as Mexico, Germany, USA, Sweden, Denmark and Zimbabwe, China, Korea and Japan (Mnkeniet al. 2008; Steinecket al. 1999). However, according to the Water Research Commission (2006), even faeces from healthy humans contain live viruses, most of which are plant viruses that could sicken and distort plants.

The organisms commonly used as the indicator of fecal contamination are certain commensally intestinal bacteria of animals (Muneeret al., 2001;) as they are always present in the feces of man and warm blooded animals intestine, their presence in drinking water and vegetables indicates fecal coliforms and parasites. In order to reach the UN Millennium objective on sanitation, ecological and conventional sanitation technologies must be developed in close association with the users (Nawabet al., 2006)

Oshikandas valley is located 24 km in the east away from the center of Gilgit city with approximately eight hundred households. The economic status of the people is not satisfactory. Eighty percent people of the area depend on their agriculture land and have a simple life style and most of the economy of people comprises on agriculture. Most of the people are related with Government and NGO while very few with business. The climate of this area is generally cold in winters and hot in summers and varies in the regions according to altitudinal differences. Based on these cultural and climatic variations the inhabitants of the area have adopted different methods of agriculture. In Baltistan, Hunza and in some parts of Gilgit where people from Hunza are settled use

contents of pit latrine as a fertilizer for crops especially for vegetables due to its enrich gradients. While in remote areas of Gilgit people use cattle sheds and open fields for defecation. The practices adapted for composting process are not as defined by scientific ways. Therefore the content of pit latrine is potential source of spreading of gastrointestinal diseases because most of the intestinal parasites and pathogenic bacteria have fecal oral route of transmission (Hussain et al., 2014).

Sanitation related diseases are the commonest ailment which is encountered by every individual at least once a year (Hafiz et al., 1991). An unpublished data shows that males are much more prone as compare to females to the sanitation related diseases. There are many other sources of fecal bacteria (*Salmonella*, *Shigella* and *E.coli*) and parasites (*Ascaris lumbricoid* (Al), *Trichuris trichuria* (Tt), *Giardia lamblia* (Gl) and *Cryptosporidium* (CRYPTO)) such as rodents, wild animals, contaminated feeds and birds. In this area people still are of the notion and belief that these un composed human feces is considered a valuable nutrient source for crop production. There are also number of health risk related to microbiological contamination that arise from drinking contaminated water or consuming contaminated vegetables due to human feces used as organic manure (Fewtrell et al., 2002). The organisms commonly used as the indicator of fecal contamination are certain commensal intestinal bacteria of animals especially *E.coli*, *Salmonella*, *shigella*, *streptococcus faecalis*, *clostridium perfringens* (Muneeret al., 2001;) as they are always present in the feces of man and warm blooded animals intestine, their presence in soil, drinking water and vegetables indicate fecal contamination (Lechevallient et al., 1990).

This study will generate some data on the physical and microbiological quality of the human manure used as a fertilizer in the fields and its possible harmful impacts on human health. If the feces of human are used as an

organic fertilizer, it must be insured that it is properly composted and is free with the human pathogenic microorganisms and fertility of soils are not negatively affected in long term prospective.

Materials and methods

Sampling

Samples were taken from pit latrine and agriculture soil amended with organic manure to check the difference in microbial load in pit samples and soil samples. During this study overall 100 samples were taken 50 samples each from pit and agriculture field soil during October 2012-July 2013, from Oshikandas valley, Gilgit (Fig.1).

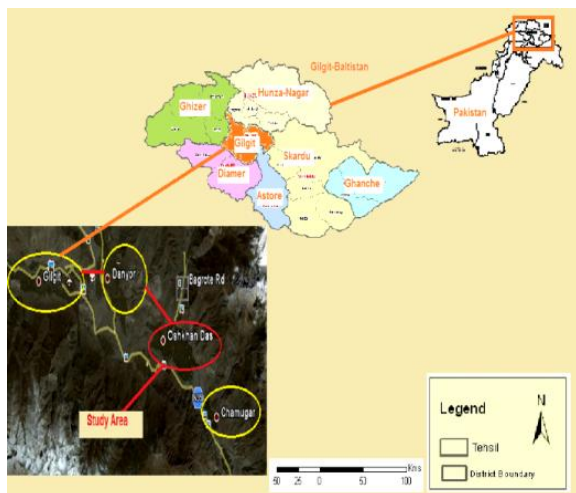


Fig.1. Map showing study area and their location.

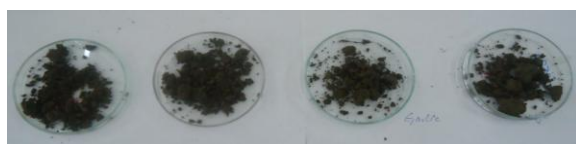


Fig.2. Soil samples in Petri dishes before weighting.

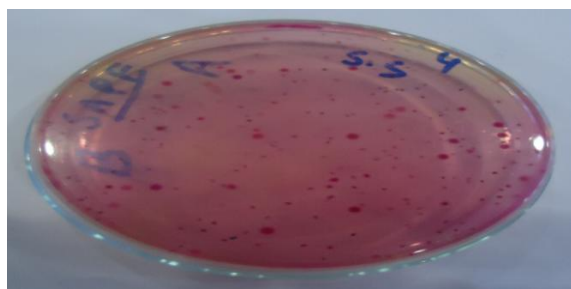


Fig.3. Petri dish for bacteria count.

Preparation of Media

MacConkey Agar: The gradient formula followed were including, Peptone 20.0 g/L, lactose 10.0 g/L, Sodium chloride 5.0g/L, Neutral red 0.075g/L, Bile salts 5.0 g/L and Agar 12.0 g/L respectively.

Glassware were washed with tape water, dried in oven and sterilized in a hot air oven at 121° C for 15 minutes. Suspended 52 g in 1 liter of distil water, brought to the boil to dissolve completely, sterilized by autoclaving at 121°C for 15 minutes, dry surface of the gel before inoculation.

Salmonella-Shigella Enrichment agar: The gradient formula followed were including, Lab limco powder 5.0 g/L, Peptone 5.0 g/L, Lactose 10.0 g/L, Bile salt 8.5 g/L, Sodium citrate 10.0 g/L, Sodium thiosulphate 8.5g/L, Ferric citrate 1.0 g/L, Brilligentgreen 0.00033g/L, Neutral red 0.025 g/L and Agar 15.0g/L respectively.

Suspended 63g in 1 liter of distill water, brought to the boil with frequent agitation and allowed to simmer gently to dissolve the agar, didn't autoclave and cooled to about 50 °C, mix and poured into Petri dishes.

Triple sugar iron Agar: The gradient formula followed were including, Lablimco powder 3.0g/L, Yeast extract 3.0g/L, Peptone 20.0g/L, Sodium chloride 5.0g/L, Lactose 10.0g/L, Sucrose 10.0g/L, Glucose 1.0g/L, Ferric citrate 0.3g/L, Sodium thiosulphate 0.3g/L, Phenol red 0.0-24g/L and Agar 12.0g/L respectively. Suspended 65 gram in 1 liter of distill water, brought to the boil to dissolve completely, well mixed and distributed into container, sterilized by autoclaving at 121 °C for 15 minutes and allowed to set as slop with 2.5 cm butts.

Sim Citrate: The gradient formula followed were including, Megnisum sulfate 0.2g/L, Ammonium dihydrogen phosphate 0.2g/L, Sodium citrate 0.8g/L, Sodium chlorite, 5.0 g/L, Bromothimol blue 0.08 g/L and Agar 15.0 g/L respectively. Suspended 23 gram in

1 litre in distill water, brought to the boil to dissolve completely, sterilized by autoclaving at 121 °C for 15 minutes allowed to set as slops with 25 cm butt.

Nutrient Agar: The gradient formula followed were including, Lab limco powder 1.0g/L, Yeast extract 2.0g/L, Peptone 5.0g/L, Sodium chloride 5.0g/L and Agar 15.0g/L respectively. Suspended 28 gram in 1 litre of distill water, brought to the boil to dissolve completely, sterilized by autoclaving at 121 °C for 15 minutes and poured into Petri dishes and allowed to cool down into room temperature.

Sensitivity test agar: The gradient formula followed were including, Lab limco powder 1.0g/L, Yeast extract 2.0 g/L, Peptone 5.0g/L, Sodium chloride 5.0g/L and Agar 15.0g/L respectively. Suspended 31.4g in 1 litre of distill water, brought to the boil to dissolve the agar completely, sterilized by autoclaving at 121° C for 15 minutes. After autoclaving poured the media into Petri dishes and allowed to cool at room temperature

Preparation of Sodium Chloride Saturated Solution: We crushed the rocky salt into powder and mixed it in 1000 ml distill water until the solution becomes completely saturated.

Enumeration of bacteria from pit latrine and soil sample

Collection of pit latrine and soil sample

Compost pit latrine and soil samples were collected from selected pit latrines and agriculture field of the Oshkhandas valley on monthly basis from Oct.2012- July 2013.

All the collected samples were packed in sterilized plastic containers and mark the identification number and transport to laboratory for laboratory investigation.

Bacterial Investigation

Serial dilution techniques

One gram of pit content was mixed in 10 ml sterilized distilled water and homogenized by vortex and was

serially diluted up to 10^1 , 10^2 , 10^3 , 10^4 , 10^5 , 10^6 and 10^7 to enumerate the bacteria. From the 10^7 one ml sample poured into selective media (MacConkey and *Salmonella-Shigella* enrichment agar) streaked with sterilized loop and incubated at 37 °C for 24 hours and read the plates.

Enumeration and Identification of targeted bacteria

The colonies were enumerated and the targeted bacteria (*E.coli*, *Salmonella* and *Shigella*) were identified by setting Biochemical tests.

Assessments of parasites from pit latrine and agriculture soil sample

Same collected samples were processed for parasitological investigation. One gram of sample was mixed in saturated sodium chloride solution and mixed thoroughly to homogenize. A plane glass slide was placed on the surface of the plastic small case and poured the saturated NaCl solution in the mixture till it touched the surface of the slide. Picked the slide and observed under the microscope for identification and enumeration of parasites in per field

Statistical analysis

All the statistical computations were carried out using SPSS software version 2012

Results

Table I shows that during the whole study period over all 100 samples (50 from pit and 50 from their soil) were processed from 10 representative houses to study the parasitic and bacterial load respectively. The parasitic load of targeted parasites (*Ascarislumbricoid*, *Trichuristrichuria*, *Giardia lamblia* and *Cryptosporidium*) in pit content was highest in house number H-524 (226.30) followed by H-734 (206.62), H-618 (203.31), H-579 (186.32), H-134 (183.98), H-37 (179.98), H-704 (175.97), H-600(171.64), H-407(153.21), H-129 (133.99) and population load of parasites in soil was highest in house number H-734 (204.29) followed by H-524 (201.43), H-618 (188.54), H-579 (176.43), H-134

(169.76), H-037 (164.30), H-600 (162.07), H-704 (161.89), H-407 (142.22) H-129 (121.01).

Table 1. Summary of overall microbial load from October 2012 to July 2013.

House No.	Parasitic Load Counted per gram		Bacterial Load Counted (CFUs)per gram		Total
	PIT	SOIL	PIT	SOIL	
	Oct.2012-Feb. 2013	March-July 2013	Oct.2012-Feb. 2013	March-July 2013	
H-129	133.99	121.01	203.63	146.61	605.24
H-134	183.98	169.76	163.29	132.42	649.45
H-037	179.98	164.30	190.27	156.54	691.10
H-407	153.31	142.22	193.27	150.98	639.79
H-524	226.30	201.43	185.96	138.99	752.68
H-579	186.32	176.43	182.61	155.74	701.11
H-600	171.64	162.07	200.62	172.30	706.63
H-618	203.31	188.54	199.29	155.42	746.56
H-704	175.97	161.89	194.95	148.41	681.22
H-734	206.62	204.29	203.95	176.88	791.74
TOTAL	1821.42	1691.95	1917.84	1534.29	6965.50

While the targeted bacterial load (*E. coli*, *Salmonella* and *Shigella*) in pit was highest in the house number H-734 (203.95), H-129 (203.63), H-600 (200.62), H-618 (199.29), H-704 (194.95), H-407 (193.27), H-037 (190.27), H-524 (185.96), H-579 (182.61), H-134 (163.29) and population load of bacteria in soil was observed highest in the house number H-734 (176.88), H-600 (172.30), H-037 (156.54), H-579 (155.74), H-618 (155.42), H-407 (150.98), H-704 (148.41), H-129 (146.61), H-524 (138.99) and H-134 (132.42).

In Fig. 4, out of over all 100 samples (50 from pit and 50 from their agriculture soil) the microbiological load in pit content samples was 3739.3 (54%) as compared to soil where the microbiological load was 3226.24 (46 %).

Fig. 5 shows percentage of parasitic load in 50 samples of pit versus 50 samples of agriculture soil. Where it is observed that the parasitic population in pit was 1821.42(52%) than in soil samples 1691.95 (48%).

Fig. 6 shows percentage of bacterial load in 50 samples of pit versus 50 samples of agriculture soil. Where it is observed that the bacterial population in

pit was higher i.e. 1917.84 (56%) than in soil samples 1534.29 (44%).

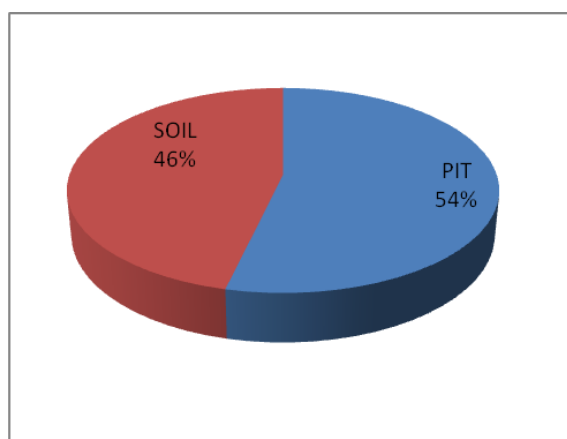


Fig.4. Overall microbial load in pit and soil samples from October 2012 to July 2013.

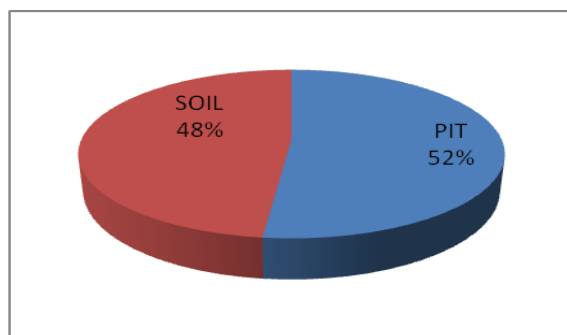


Fig. 5. Overall parasitic load in pit and soil samples from October 2012 to July 2013.

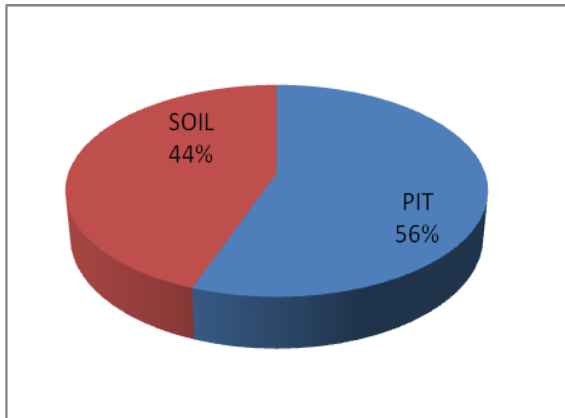


Fig. 6. Overall bacterial load in pit and soil samples from October 2012 to July 2013.

Fig. 7 shows total percentage of each parasite in 100 samples (CRYPTO) (50 samples in pit and 50 in soil). Among hundred samples the percentage of individual parasite was observed i.e. *Ascaris lumbricoid* (Al) 2395.53 (68%), *Trichuris trichiuria* (Tt) 255.24 (7%), *Giardia lamblia* (Gl) 294.98 (9%) and *Cryptosporidium* (CRYP) 567.58 (16%) respectively.

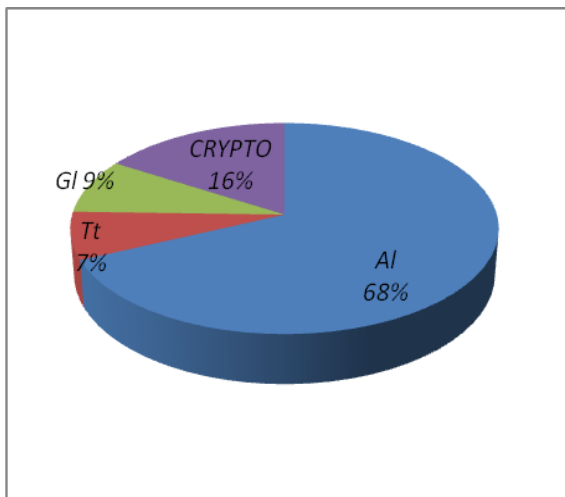


Fig. 7. Total population of targeted parasites in pit content and soil samples from October 2012 to July 2013.

Fig. 8 shows total percentage of each bacteria in 100 samples (50 samples in pit and 50 in soil). Among hundred samples the percentage of individual bacteria was studied i.e. *E. coli* 1856.40 (54%), *Salmonella* 1119.54 (32%) and *Shigella* 476.19 (14%) respectively.

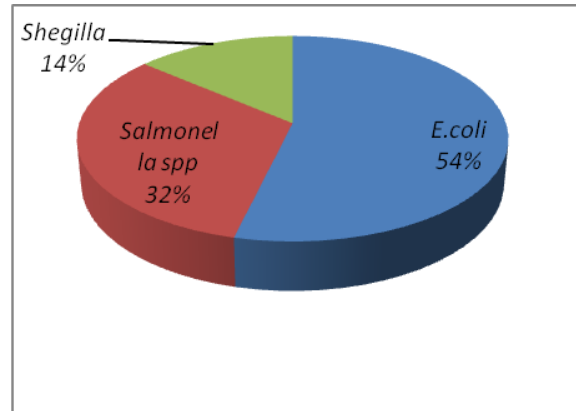


Fig. 8. Total population of targeted bacteria in pit and soil samples from October 2012 to July 2013.

Discussions

This study was carried out to find the health risk of pit latrines being used as traditional toilet and its content used as organic fertilizer to grow crops in agriculture field in Oshkhandas valley Gilgit-Baltistan, Pakistan. Throughout this study ten houses were selected for sampling from pit and ten agriculture fields were selected for soil samples to study the difference in microbial load during Oct. 2012 to July 2013. Samples were carried out after taking the consent of pit owners and agriculture field owners. During this study overall 100 samples were taken 50 samples each from pit and soil.

The study was conducted to evaluate the impact of pit latrine on human health. In Oshkhandas village people still use traditional pit latrines content for their crops production due to which the health and hygiene system of the area is not up to the mark. Most of the dwellers rely on their agriculture production and its income to meet the demands of life. To fulfill their food and other demands the farmers of the area use pit latrine content as natural organic fertilizer for their crop production

It is evident that parasites and bacteria are the potential thread to the human beings and cause many diseases where they harbor. These parasites and bacteria are also major source of communicable diseases (Vuong, 2004). It has also been observed

that the population of parasites or parasitic load and bacterial load in pit latrine or in area where people practice open human defecation depend upon many factors such, sanitary facility, population load, socioeconomic status of people, availability of portable water, environmental condition, personal hygiene and temperature (WHO/UNICEF, 2006). Our study revealed high prevalence of parasites and bacteria in the pit and soil sample where the population burden was high, lack of basic health facilities with poor environmental condition and suitable temperature for the parasites and bacteria. This research showed that the availability of sanitary facilities was inadequate in the house with high parasitic and bacterial load and this is of epidemiological significance considering the number of persons using the same pit. Besides this unavailability of clean water enhanced the rate of microbial load. This study also came to the point that poverty is the one of the major root causes of microbial load and its infection and our study appeals to work on poverty elevation where people suffer from the pathogenic organisms. This will consequently minimize parasitic and bacterial disease transmission among people. Our study came to this point that serious heed must be given to improve the sanitary facilities in rural areas where still pit latrines are being used and people must be taught personal hygiene ethics through health education. This will in the end minimize the bane of gastro-intestinal parasites in the human beings. Our observation links to Murray and Lopez 1996; where they stated that Human waste and meagerness of personal and domestic cleanliness have been considered in the spread of various infectious diseases including cholera, hepatitis, *Ascariasis* and *Cryptosporidiosis*. The world health organization (WHO) estimated that 2.2 million persons succumb to death yearly due to diarrheal diseases while 10% of the population of the developing world is rigorously infected with intestinal worms due to ill waste and excreta management (Murray and Lopez, 1996).

In the same way as parasites and bacteria were analyzed in pit when the parasites and bacteria were studied in soil samples it showed the meagerness in their population in agriculture soil as compared to pit samples. During this study when overall microbiological load in pit versus soil was studied from the month of October 2012 -July 2013 the population load of microbes were seen 54% in pit and 46% in soil. And when total population of parasites in Pit versus soil was studied it appeared to be 52% in pit and 48% in soil. When bacterial load was examined in pit versus soil the bacterial load in pit were observed to be 56% and 44% in soil. As studied above when the total population of targeted parasites (*Ascaris lumbricoid* (Al), *Tricuristricurica* (Tt), *Gardialamblia* (Gl) and *Cryptosporidium* (CRYPTO) in the pit and soil in the area were studied they were seen Al =48%, Tt=7%, Gl=9% and CRYPTO=16% respectively. When the overall bacterial load in the area i.e. in pit plus in the soil were counted they were found to be *E.coli* 54%, *Salmonella* 32% and *Shigella* 14%. Besides, it is also revealed that the parasites and bacteria in soil were less than their population load in the pit samples. Hence we came to know that the suitable environment for microbes was in the pit where their number was seen to be high.

It is concluded that Pit latrines and organic manures are the main key determinants to groundwater contagion in Oshkhandas valley, Gilgit. Groundwater with faecal bacteria has direct approach to the epidemic of waterborne diseases (diarrhea and typhoid). The competence of health facilities is inadequate to switch cases of these outbreaks thereby necessitate capacity building. Ecosan Toilet Technology might overturn the tendency if attached with suitable awareness and local institutional capacity building. Hygiene of the targeted area needed to improve by social training, and SODIS technology may be used for safe drinking water. Epidemiological situations should be handled under the basic health principles of WHO.

Acknowledgments

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