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Research Article

Effect of Treated Sewage Water on Survival of Microbial Community and Rice Production in Northwest India

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Abstract

Huge amount of sewage water is generated every day in high populated cities of India and recycling those water as crop irrigation could potentially reduce the sewage loads in the main river's streams and other fresh water bodies. Punjab, geographically located in the north western part of India, has been exploited in terms of ground water development from last few decades. So, this two year of field study on periodic survival of fecal coliforms (FC) in secondary treated sewage water (SW) and soil, and rice yield analysis was specifically conducted at Rice Research Station (RRS), Kapurthala Punjab, India to propose the option of sewage irrigation with the aim to save some quantity of drinking water in that state. However, sewage irrigation can pose threat to human health through microbial contamination. So, our study measured the population of *E. coli* (an indicator organism) to quantify the waste water pathogens in applied water and in the soil and subsequently the suitability of the treated SW for irrigation. Fecal coliform community was measured by two methods includes the most probable number method and plate count technique. Tube well water irrigation was used as control for this study. The average FC population was found > 100 times (MPN) and > 50 times (plate count) higher in sewage flood water as compared to TW just after irrigation. However, the average population of FC in the SW decreased (in 0 to 48 hrs) by 4 and 2 times in MPN and in plate count, respectively, for two years. The soils irrigated with treated sewage water had about 26 times higher FC population than TW irrigated soils during different cycles of the study. When irrigated with different doses of sewage (3, 6, and 12 irrigations), rice production was about 50 - 60% higher than controlled plots indicates the substantial amount of nutrients provided by sewage water in the soil. The field was left to dry out for 2 weeks after rice harvesting and interestingly the difference of total coliform (TC) and FC concentration was found statistically insignificant ($P > 0.05$) in soils irrigated through TW and treated SW. Several soil and environmental factors such as high temperature, low rainfall, low soil moisture content, soil pH etc, possibly played significant roles for the survival of total coliform and fecal coliform in rice soil.

Keywords: Fecal Coliforms (FC); Most Probable Number (MPN); Plate Count; Waste Water Treatment; Treated Sewage Water Irrigation; Water Quality; Rice Yield

Introduction

The disposal of daily generated sewage water in highly populated countries like India is very challenging and often time those sewage waters are dumped into river streams, lakes, and other water bodies with or without proper treatment. The Central Pollution Control Board of India [1] estimated that only urban areas of India generate about 62,000 million litres of sewage every day, whereas, the treatment plants in India can only treat about 37% of that total sewage produced. The importance of sewage treatment for proper handling and disposal is an imperative area of concern and several Governmental schemes such as National River Conservation Plan (NRCP), National Ganga River Basin Authority (NGRBA) have been made till date to conserve the fresh water quality of rivers and lakes including one of the most popular "Save Ganga Movement" for past few decades. Currently it was reported that more than 6000 million litres of sewage water have been discharged into river Ganga in a single day through 138 sewage channels [2] which results in bacterial pathogen contamination, high biological and chemical oxygen demand for microbes (BOD and COD), and high NH_4^+ loads [3]. However, irrigating agricultural crops with reclaimed or properly treated waste water can be another option for a logical and important component of water resource planning and development in India. The limits of biological oxygen demand (BOD) and chemical oxygen demand (COD) for sewage disposal on land surfaces (BOD ≤ 100, COD ≤ 400 mg l^{-1}) is higher than disposal in the main water streams (BOD ≤ 30, COD ≤ 250 mg l^{-1}) in India [1] which basically indicates the comparative advantages of disposing the treated sewage water as crop irrigation than dumping them in fresh water bodies. Additionally, the use of treated sewage water as irrigation increase the concentrations of N, P, K, organic C, and some micronutrients in the applied soil [4] increase soil enzyme activity [5] and subsequently increase the crop productivity [6-8].

It can be pointed out that numerous numbers of pathogenic organisms can be found in untreated sewage water but most cases the treatment of sewage are capable to decrease microbial population due to the removal of sludge. Another important aspect is the accumulation of heavy metals such as Pb, Cd, Fe, Cr, Cr, etc. In food grains [3,9] and the contamination from human pathogens in waste water irrigated areas may negatively impact crop quality and human health safety [10-12]. However, for the objective of this research article, we will mainly focus the microbial aspects of sewage water irrigation and crop production. Representative genera of bacterial pathogens that can be found in waste water includes *Salmonella, Shigella, Clostridium, Vibrio, Escherichia, Campylobacter, Yersinia*, and many more. All these pathogenic bacteria have the high potentiality of transmitting diseases in human beings and generally it occurs through direct physical contact with waste water, consumption of products irrigated with sewage effluent, contaminated ground, and surface waters [13,14]. Coliform bacteria have served as indicators of fecal contamination (indicator bacteria for the presence of human pathogens) in water for many years and their densities have been utilized as the criteria for the degree of microbial water pollution. The survival period of pathogenic organisms in treated sewage water and irrigated field crop indicates the possibility of safe use of treated sewage water safe for agriculture. Pathogens in domestic waste water are often exposed to adverse soil and environmental conditions once they are introduced into the soil and are expected not to survive for extended periods of time or, to multiply mostly because they are unable to survive outside the host for longer period of time. Recent publications have shown that *E. coli* (natural pathogen in human intestine) can survive on a long-term basis in the soil after contamination [15,16] Survival of fecal coliform (FC) in soil applied with sewage mainly depends on the level of waste water treatment (primary, secondary, and tertiary), soil moisture, pH, clay content, soil organic matter content, and other factors [15,17,18] and the concentration of FC is found strongly correlated with BOD of the waste water [19-21].

Punjab, geographically located in northwest of India, is one of the major agricultural crop production states in India. However, availability of ground water in Punjab is insufficient for drinking and therefore, its excessive use for irrigation is becoming a serious water problem. It has been reported that more than 75% of the total geographical area in Punjab is over exploited in terms of stage of ground water development [22]. Basically, in this current situation of water scarcity, irrigating agricultural crops with treated waste water seems to be better possible option for handling the huge amount of sewage generated every day in India.

Punjab (India) has installed sewage treatment plants in major cities to treat the sewage for its proper disposal. Presently, six treatment plants e.g. at Bhattian, Balloke (Ludhiana), Kapurthala, Jalandhar, Phagwara, and Jamalpur (Ludhiana) are fully operational and more plants are expected to be installed in various cities in near future. These plants are expected to produce large quantity of nutrient-rich treated water that may be used for the benefit of agriculture. The sewage treatment plant at Kapurthala, Punjab, India operated based on the principle of on Upflow Anaerobic Sludge Blanket technology (UASB). Rice is one of the main grain crops in India (June to October) and generally grown in submerged water condition which actually causes a huge amount of water loss through percolation, seepage, and field preparation. Punjab itself produced more than 11 million tonnes of rice during 2014 - 2015 [23] which clearly required huge amount of water for production. So, being a proliferate user of water, regular rice production becomes one of the major reasons for physical and economical water scarcity in India (Sita Devi and Ponnarasi, 2009). Thus, quantifying the FC organisms in the treated waste water and irrigated soil will help us in understanding the suitability of that water for rice irrigation. Therefore, this research study was planned with the objectives of (1) to study the effect of treated sewage irrigation on yield of rice and (2) to analyze the periodic survival of coliform viz. a viz. pathogenic organism in rice flood water and in soils irrigated with secondary treated waste water.

Materials and Methods

Site location and characteristics

The field experiment was carried out at Rice Research Station (RRS) of Punjab Agricultural University (PAU) located in Kapurthala (31°23′N 75°23′E), Punjab, India. Kapurthala is one of the high populated cities in Punjab, and the sewage water generated in Kapurthala is mainly from different industrial byproducts and little portion from human inhabitation (domestic). The RRS is basically located in the vicinity of one sewage treatment plant which made easier for us to collect secondary treated sewage water for this study. The experiment was conducted on a non-saline (EC = 0.36dS m^{-1}), alkaline (pH = 8.5) clay loam soil, with low available nitrogen (75 kg N ha^{-1}); medium organic carbon content (OC = 0.5%) and high amount of available phosphorous (29.4 kg P ha^{-1}) and potassium (300 kg K ha^{-1}) contents. The average annual rainfall of this research station is 779 mm, major amount of which is received in during monsoon seasons (July and August).

The Field experiment was carried with the following treatments (3 replications each) in a Randomized Complete Block Design (RCBD). However, these treatments were only used to see the yield component, individual treatments were not used to quantify FC population in soil or water.

- T1= Irrigation with tube well water + No N
- T2= Irrigation with tube well water + 120 kg N ha^{-1} (recommended N to rice)
- T3= Irrigation with tube well water + 80 kg N ha^{-1} (67% of recommended N to rice)
- T4= Irrigation with tube well water + 40 kg N ha^{-1} (33% of recommended N to rice)
- T5= 3 irrigations with sewage water and the remaining with tube well water
- T6= 6 irrigations with sewage water and the remaining with tube well water
- T7= 12 irrigations with sewage water and the remaining with tube well water.

Layout of the experiment and the scheme for application of tube well and treated sewage water is presented in figure 1. Ten cm periodic irrigations were applied as per the crop requirement. Tube well water was used as control in this experiment. The irrigation with both sources of water was applied at the same time and the experiment was conducted for two years.

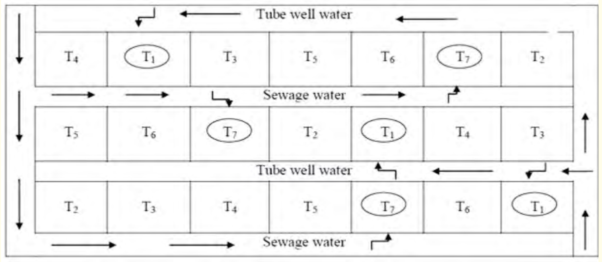

Figure 1: *The schematic layout of the experimental field plots at RRS, Kapurthala, Punjab, India.*

Sample collection

Two treatments i.e. tube well water (T1) and treated sewage water (T7) irrigated field plots were selected for collection of water and soil samples for this study (Figure 1). Five random points were chosen for collection of samples from the SW and TW irrigated plots to determine the FC and total coliform population of soil and the applied water. Flood water samples from each point in the plot were collected with the help of 100 ml pre-sterilized beakers and stored in the sterilized bottles immediately after collection. The bottles were then kept in ice-chest for transportation to the laboratory. Surface soil samples were collected from each plot by scooping top 0 - 5 cm and composite samples were stored in polythene bags each time before and after the application of irrigation. The sampling was done three times in cycles (cycle I, II, and III) during July to October in both years of this study. Each cycle was composed of collecting water samples at 0, 24, and 48 hrs after irrigation. Water samples at 0 hrs were collected immediately after the irrigation was applied. The experimental plots were left to dry for 5 days in between each cycle. The basic idea of collecting samples within 48 hours of irrigation and left it dry for 5 days is to simulate the alternate drying and wetting process of rice cultivation which has been adapted globally to increase water use efficiency. The samples were always analyzed within 24 hours of collection and composite soil samples were kept overnight (24 hours) in an air-hood for air drying.

Laboratory analysis

Chemical parameters of the collected treated sewage water samples used for irrigation were analysed for biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total dissolved solids (TDS) by standard methods [24]. Electrical conductivity (EC) and pH of the treated sewage water samples were determined by using conductivity bridge and pH meter fitted with glass electrode, respectively (Ayers and Mara, 1996). Average values (n = 8) of physiochemical properties of the treated sewage water were as follows: pH 7.0 ± 0.3; EC 1.0 ± 0.2 dS m^{-1}; BOD 43.0 ± 6.7 mg l^{-1}; COD 140 ± 21 mg l^{-1}; TS 720 ± 187 mg l^{-1}; TDS 524 ± 194 mg l^{-1}; and TSS 196 ± 63 mg l^{-1}. Same physiochemical parameters were also determined for the tube well water as reported here, pH 7.2; EC 0.31 dS m^{-1}; BOD 8 mg l^{-1}; COD 22 mg l^{-1}; TS 262 mg l^{-1}; TDS 216 mg l^{-1}; TSS 46 mg l^{-1}.

Most Probable Number (MPN) of FC in rice flood water

The concentration of FC bacteria was detected by most probable number (MPN) method (Mackie and McCartney, 1996). Three double strength and six single strength Mc-Conkey broth tubes were taken. Water samples were mixed thoroughly and inoculated aseptically into the dilution tubes. Three tubes of double strength medium were inoculated with 10 ml water sample and three tubes of single strength were inoculated with 1 ml and rest three tubes were inoculated with 0.1 ml of water sample. The tubes were incubated at 44ºC for 48 hrs. The formation of gas and acid confirms the presence of FC, as demonstrated by the change of the medium color from purple to yellow. And the results were interpreted using the standard 3-tube MPN table.

The samples showing positive results were streaked onto a selective differential medium, Eosin Methylene Blue (EMB) agar to confirm the above test. The positive test is shown by the appearance of typical coliform colonies with dark centers and green metallic sheen.

Plate counting of FC and total coliform count

The population of FC in soil and water was determined by standard pour plate technique using EMB agar plates (Mackie and McCartney, 1996). Ten grams of air-dried soil sample or 10 ml of the water sample was diluted in 90 ml of sterile water blanks and shaken for 30 minutes on a mechanical rotary shaker at 160 rpm. One ml from dilution blank was pour plated and the plates were incubated at 44ºC for 48 hrs for enumeration of colonies per ml of the sample. The counts were calculated on soil dry weight basis using moisture factor.

The total coliform population in air dry soil samples collected at the harvest of the crop was estimated by pour plate technique using EMB agar [25]. The population of total coliforms was estimated by incubating the plates at 350C for 48 hrs. The counts were calculated on soil dry weight basis using moisture factor.

Statistical Analysis System (SAS 9.4; North Carolina, USA) software was used to run the ANOVA (5% significance level) to compare the mean differences in plate count, MPN number of fecal coliforms in TW and SW samples and soil samples irrigated with different water treatments.

Results and Discussion

Most Probable Number (MPN) and Plate count of Fecal coliforms (FC) in rice flood water

The most probable number analysis of coliform organisms present in rice flood water is presented in table 1. Tube well water (TW) irrigation was considered as control for this experiment and average MPN numbers were found 1.01 x 101 (100 ml^{-1}) in first year and 0.94 x 101 (100 ml^{-1}) in second year. The total coliform (TC) content in safe drinking water (tube wells, hand pumps etc.) was reported lower (generally 3 - 10 MPN per 100 ml water) than our TW values in several soil microbiological studies conducted in India [26,27]. It can be pointed out that the tube well water used for this research study was mainly used for irrigation purposes, not for drinking. As expected, the MPN of FC in rice flood water irrigated by treated sewage water (SW) were significantly higher ($P < 0.001$) than TW as measured in different cycles (cycle I, II and III) and times (0, 24, and 48 hours). The average FC counts (for all cycles) in SW was found more than 100 times higher than SW irrigation over the years. However, on an average the MPN count of SW was more than 160 times higher than TW for 0 hours after irrigation. But when we measured the MPN count in SW water after 48 hours of irrigation, it was about 40-45 times higher than MPN count of TW water. And interestingly some of those FC values in TW and SW were not statistically significant ($P > 0.05$) from each other. This is possible because the 48 hours' time period diluted the FC population in the irrigation sewage water and most of those FC organisms did not performed well outside of a host body. Though we did not measure the environmental and meteorological factors, but studies indicate the importance of these factors for reducing the amount of FC over time [28,29]. Overall, the year 1 and year 2 did not show any significant difference in MPN count for both TW and SW irrigations.

Sample		Cycle I			Cycle II			Cycle III	
	0 hrs	24 hrs	48 hrs	0 hrs	24 hrs	48 hrs	0 hrs	24 hrs	48 hrs
					YEAR 1				
TW	0.94 ± 0.07a	1.31 ± 0.08a	1.13 ± 0.11a	1.11 ± 0.11a	1.01 ± 0.19a	0.89 ± 0.08a	1.01 ± 0.14a	0.81 ± 0.09a	0.93 ± 0.07a
SW	18.95 ± 1.72b	14.59 ± 0.86b	4.79 ± 0.47a	14.94 ± 1.01b	10.16 ± 0.61b	3.06 ± 0.49a	14.95 ± 0.99a	7.94 ± 0.64b	3.79 ± 0.25c
					YEAR 2				
	0.98 ± 0.12a	1.01 ± 0.09a	0.93 ± 0.07a	1.01 ± 0.11a	0.91 ± 0.09a	0.89 ± 0.09a	0.94 ± 0.13a	1.01 ± 0.12a	0.83 ± 0.07a
SW	15.41 ± 1.60a	11.51 ± 1.01a	4.29 ± 0.47b	14.94 ± 0.93b	9.16 ± 0.81b	3.79 ± 0.41a	15.95 ± 0.99a	11.94 ± 0.64a	4.79 ± 0.65b

Table 1: *Most probable number (MPN) analysis of the tube well water (TW; x 101 per 100 ml) and treated sewage water (SW; x 102 per 100 ml) irrigations at Regional Rice Research Station of Kapurthala, Punjab, India.*

Plate counts of FC from flood water samples (TW and SW) are presented in table 2. Plate count (cfu) of TW was very consistent throughout different cycles and in both years. It ranged from 1.40 to 3.14 CFU, whereas FC population varied greatly, specifically between hours after irrigation was applied. The average amount of FC population in SW was more than 50 times higher than TW. It was interesting to see that the magnitude of plate count quantity SW was reduced more than 1.8 times within 48 hours after irrigation was applied. Overall, the amount of FC was significantly higher ($P < 0.001$) in SW immediate after irrigation, but in couple of cases (cycle III for both years) the difference in amount of FC between SW and TW was minimal. It is possibly because after a prolonged period of time the amount of FC in irrigation water reduced in SW irrigation.

Sample		Cycle I			Cycle II			Cycle III	
	0 hrs	24 hrs	48 hrs	0 hrs	24 hrs	48 hrs	0 hrs	24 hrs	48 hrs
					YEAR 1				
TW	2.95 ± 0.22a	2.01 ± 0.21a	2.03 ± 0.27a	3.14 ± 0.31a	2.51 ± 0.33a	2.03 ± 0.19a	2.94 ± 0.24a	2.30 ± 0.27a	2.02 ± 0.30a
SW	15.84 ± 1.72a	11.94 ± 0.86ab	8.03 ± 0.47b	19.95 ± 1.01a	13.01 ± 0.81b	9.04 ± 0.49c	16.84 ± 0.99a	10.00 ± 0.64b	7.03 ± 0.25b
					YEAR 2				
TW	2.14 ± 0.12a	2.94 ± 0.26a	1.40 ± 0.17a	3.12 ± 0.31a	2.94 ± 0.29a	2.08 ± 0.39a	1.95 ± 0.33a	1.94 ± 0.27a	2.08 ± 0.30a
SW	14.91 ± 1.30a	9.84 ± 0.75b	8.06 ± 0.47b	17.81 ± 2.93a	14.58 ± 0.81a	1.10 ± 1.01a	18.61 ± 1.29a	13.58 ± 0.64b	7.10 ± 0.65c

Table 2: *Plate count of FC (CFU) in tube well water (TW; x 101 ml-1) and treated sewage water (SW; x 102 ml-1) irrigations at Regional Rice Research Station of Kapurthala, Punjab, India.*

Another important aspect is the time after irrigation which influenced the FC population in SW. For instance, the amount of FC in TW after 0 hours was always significantly higher ($P < 0.05$) than 48 hours (except cycle II in second year) indicating the relative disadvantage of survival of those fecal organisms outside of a host.

Fecal coliform (FC) population in soils irrigated with TW and SW

Fecal coliform population (plate count) in soils irrigated with TW and SW were analysed to examine their survival in the experimental soil after cycle III of irrigation (Table 3). Fecal coliform population in SW-soil was more than 25 times higher than TW-soil for two-year study period. Tube well-soil had very consistent FC population ($P > 0.05$), however it was interesting to see that SW-soil had significantly lower ($P < 0.05$) FC in cycle III as compared to cycle I for both years. After the irrigation was applied in different cycles, the field was allowed to dry for harvesting and it took about 14 days to dry completely during October. Immediately after harvesting of rice, the composite soil samples were collected to measure the population of total coliform (TC) and FC in TW and SW irrigated plots. The amount of TC and FC in SW irrigated soil was not significantly different ($P > 0.05$) than TW at the harvesting stage of rice for both years (Figure 2). However, TC count of SW and TW irrigated soils were significantly higher than FC populations. It can be concluded that after prolong period of irrigation application, fecal organisms have low possibility of survival in the soil, specifically during hot weather and low soil moisture content.

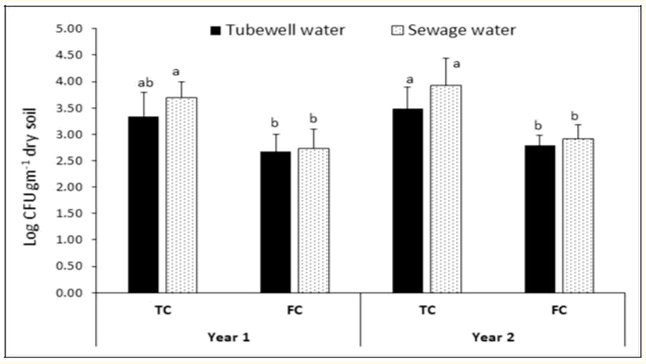

Figure 2: *Total coliform count (TC) and plate count of fecal coliforms (FC) of sewage and tube well water irrigated soils after rice harvesting.*

Sample		Year 1			Year 2	
	Cycle I	Cycle II	Cycle III	Cycle I	Cycle II	Cycle III
TW-Soil	0.89 ± 0.11a	1.09 ± 0.18a	1.05 ± 0.17a	1.01 ± 0.11a	0.96 ± 0.14a	1.09 ± 0.09a
SW-Soil	4.16 ± 0.73a	2.26 ± 0.27b	1.58 ± 0.25b	5.02 ± 0.81a	3.16 ± 0.51ab	1.16 ± 0.11b

Table 3: *Plate count of FC in tube well (TW-soil; x 102 cfu g-1 dry soil) and treated sewage irrigated (SW-soil; x 103 cfu g-1 dry soil) soils at Regional Rice Research Station, Kapurthala, Punjab, India.*

Although the waste water treatment did not remove all the indicators, the data are in accordance with the typical percentages of microbial inactivation [30]. The decrease in the population of FC indicates that the pathogenic load is reduced in soils at the time of harvesting. The TC in the SW irrigated soils was higher even in the end. The addition of mineral nutrients and organic matter may favor high bacteria count in SW irrigated soil. Organic matter is very important for water retention, the formation and stabilization of aggregates and the formation of microhabitats, all factors which have a strong influence on the survival of micro-organisms in the soil. The decrease in the population of coliforms in our study can be attributed to the fact that the sunlight and high temperature during July-October (The average temperatures in July, August, September and, October were 37.1, 33.9, 33.5 and, 32.5 0C, respectively) in the field lowered the survival of FC in the flood waters over time after irrigation. Favorable temperature generally helps the survival of bacteria in soil with survival levels tending to decrease as the temperature increases. Garcia-Orenes., *et al.* [29] reported that the presence or absence of irrigation is the most important factor for the growth of total coliform bacteria.

The absence of moisture led to sharp decrease in the number of total coliforms (fresh sewage sludge added at 50g kg^{-1} soil) in soils with the bacteria disappearing in 40 days. Even with the addition of water the coliforms disappeared in 80 days [29]. Estrada., *et al.* [31] documented that after 20 days of incubation of sewage sludge treated soil, the pathogenic microorganisms of fecal origin went below detectable limits.

The persistence of enteric bacteria in aquatic environment depends upon the species and different environmental factors like temperature, pH, sunlight, competition by aquatic microorganisms, dissolved organics, and association of vectors and presence of salts/solutes. The decrease in the population of coliforms in our study may be attributed to the fact that the sunlight and high temperature during July-October in the field lowered the survival of coliforms in the flood water irrigation [28]. Favourable temperature helps the survival of bacteria in soil with survival levels tending to decrease as the temperature increases. A low incubation temperature and high soil moisture aid the survival of *E. coli* and *Enterococcus* sp [32]. Seasonal variations have a marked effect on the bacterial quality of water [33] as the survival of coliforms is more during rainy season due to favourable temperature and high turbidity. Another reason of the decreasing coliform population is due to the pH of the sewage water (slightly acidic pH) used for irrigation at Kapurthala, India.

The total bacterial population in the sewage irrigated soil was higher even in the end. The decrease in the population of fecal coliforms indicates that the pathogenic load got reduced below the permissible levels in soils after harvesting. However, the possible accumulation of pathogens in soils and their entering into food chains due to irrigation with sewage effluent at later stage cannot be disregarded [34].

Yield analysis of rice

Grain yield of rice (kg ha^{-1}) with different nitrogen applications (40, 80, and 120 kg N ha^{-1}) were compared with the yield obtained by three types of sewage irrigations (3-irrigations, 6-irrigations, and 12-irrigations) for two years (Figure 3). Average rice yield was found 7% higher in year 2 as compared to first year possibly because little bit higher rainfall was received for second year. On an average, rice yield was increased by 50 - 60%, with different sewage water irrigation (SWI) as compared to the yield obtained in controlled plots. Saha., *et al.* [35] reported similarly that average grain and straw yield of cereal crops were increased 31% and 43%, respectively, with sewage water application to the field. When we used 12 or 6 SWI, it produced statistically higher yield as compared to 40 kg ha^{-1} N application. No yield difference was found with application of 80 kg N ha^{-1} and 6 or 12 SWI. But yield obtained with N application rate of 120 kg ha^{-1} was statistically higher than yield obtained with 6 SWI ($P < 0.001$) or with 12 SWI ($P < 0.001$). It indicates that application of N at 120 kg ha^{-1} is the best treatment to achieve the highest grain yield. But rice yield was not statistically different with 6 SWI and 12 SWI ($P > 0.01$) which depicts that irrigation with 6 sewage water can be much more cost effective and can produce significant rice yield. Also, with the 12 SWI, the excess water can be lost from the field or adversely affect the crop quality. A common factor of reduced rice yield even after higher dose of fertilizer and sewage irrigation (provide different nutrients in soil) because of loss of applied N as ammonia [36] or N_2O from soil surface [37,38]. Our result clearly indicates that secondary treated sewage water supplies a very good amount of nutrients even after which can increase the rice yield significantly with very low possibility of microbial contamination in the soil.

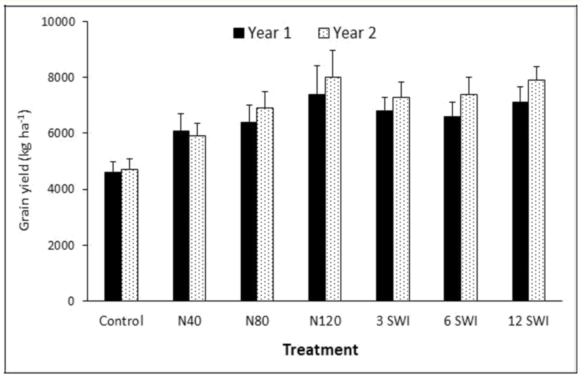

Figure 3: *Grain yield of rice (kg ha-1) with different N application rates (40 kg, 80 kg, and 120 kg ha-1) and sewage water treatments (3, 6, and 12 sewage water irrigations) two years of the study.*

Conclusions

The main goal of this study was to compare the concentration of FC in tube well water, sewage water and soils where TW and SW irrigation was applied over a two-year study period. Another important aspect was to see the effect of those irrigation sources on rice yield. As expected, fecal coliform population in treated SW was found much higher than TW throughout the rice growing season for both seasons. Also, the results showed that the FC concentration was highest in 0 hours (for all the cycles) after irrigation and become decreasing with 24 and 48 hours of irrigation application. Total FC populations at the harvesting stage of rice were statistically higher in SW as compared to TW. But population of FC in SW irrigated soil was not significantly different than the soils irrigated with TW at harvesting stage of rice. Thus, soil with SW irrigated field at crop harvesting stage has significantly lower population of coliform as compared to the soil samples collected at very early stage of rice cultivation. It is possibly because the desiccation of the soil resulted in a decrease in the population of FC in SW irrigated soils for both the crops. However, more interestingly we found that the population of those FC organisms were very similar (not significantly different) even after very short period of irrigation was applied.

It clearly indicates that those FC cannot perform well outside of a host, and their contamination in soil and food grains can easily be avoided after secondary treatment and over time in the field. It should also be noted that that different soil and environmental factors such as temperature rainfall, soil moisture content, etc. can play a major role for FC survival in the soil and sewage irrigation water. This research study specifically addressed the question of the fate and risk of FC population in soil and water after a shorter period of irrigation (48 hours) which has not been adequately investigated in the past. However, our study did not able to characterize the microbial species (qPCR or microbial gene sequencing) in those sewage irrigation water due to unavailability of those research instruments and facilities, still it shows the possibility of recycling secondary treated sewage water in the field as irrigation and obtain optimum yield without or with minimum health risk factor to human beings.

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