Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 6 Issue 7 July 2023

Research Article

Influence of Sewage Irrigation on Microbial Indicators of Soil Quality Under Vetiver Coverage

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DOI: 10.31080/ASMI.2023.06.1275

Received: April 26, 2023
Published: June 23, 2023

Abstract

Soil microorganisms play a vital role in maintaining soil fertility and productivity by driving most of the soil processes. The present study focused on the influence of sewage irrigation on biological activities of soil under vetiver coverage and it helps us to gain a better understanding of the use of biological properties as soil quality indicators. The study includes the assessment of soil pH, organic carbon, heavy metals, changes in soil microbial processes such as microbial biomass carbon (MBC), active microbial biomass carbon (AMBC), basal soil respiration (BSR), metabolic quotient (qCO2), total microbial activity (FDA hydrolysis) and activities of enzymes such as protease, dehydrogenase, phosphatases, peroxidase, and invertase, as it serves as potential indicators for soil quality measurement resulting from sewage irrigation in soils with vetiver cultivation. The study revealed that although there is a significant concentration of heavy metals in sewage, sewage irrigation improved the biological characteristics of vetiver cultivated soils to a good extent as is evident from the increased MBC, AMBC, BSR, FDA hydrolysis, enzymatic activities, and decreased qCO_2 in soils. The nutrient-rich sewage might have increased the microbial population and stimulated the microbial processes in the rhizosphere of the vetiver by reducing the impacts of toxic heavy metals.

Keywords: Biological Properties; Sewage; Soil Quality Indicator; Vetiver

Introduction

With an increase in population growth, the demand for natural resources to meet basic needs of humans also increases and it in-turn affects the availability and quality of existing of resources and it also increases the volume of waste water generation. One of the most important water polluting sources in India is the discharge of untreated sewage in water courses, which is surface and ground waters. Out of about 38254 million liter per day of sewage generated in class I cities and class II towns, treatment capacity exists for only about 11787 million liter per day. Thus, there is a huge gap between generation and treatment of wastewater in India. Even the treatment capacity existing is also not effectively utilized due to operation and maintenance problem. Operation and maintenance of existing plants and sewage pumping stations is not satisfactory, as nearly 39% plants are not conforming to the general standards prescribed under the Environmental (Protection) Rules for discharge into streams as per the CPCB's survey report (CPCB, 2021) [12].

Now a day wastewater use is become a common practice worldwide. As freshwater sources become scarcer, wastewater use has become an attractive alternative for conserving and expanding available water supplies. Wastewater use can have many types of applications, including irrigation of agricultural land, aquaculture, landscape irrigation, urban and industrial uses, recreational and environmental uses, and artificial groundwater recharge. Principally, wastewater can be used for all purposes for which freshwater is used, given appropriate treatment. In both developed and developing countries, the most prevalent practice is the application of sewage (both treated and untreated) to land. As there is scarcity of fresh water for irrigation and high cost for waste water treatment, promoted the use of sewage and other industrial effluents for irrigating agricultural lands (Asano., *et al*. 2007; Rai., *et al*. 2011) [6,36].

Sewage is considered most enriched with macro and micro nutrients required for the plant growth and therefore, farmers prefer the sewage irrigation for saving the cost of fertilizers and irrigation water. Accordingly nutrient levels of soils are expected to increase with continuous irrigation with sewage. Composition of raw sewage depends on the source and its characteristics. Even though the common constituents in sewage are organic matter, nutrients (Nitrogen, Phosphorus, Potassium), inorganic matter (dissolved minerals), toxic chemicals, metals and pathogens (Hussain., *et al*. 2002) [23]. Besides nutrients, heavy metals and other toxic chemicals are also present in the sewage and leads to the build-up of these heavy metals and other non-degradable toxic chemicals in soil and it eventually deteriorates the soil quality (Reddy and Rao, 2000) [39].

Several studies suggest that sewage and other waste water application to soil will cause changes in natural quality of soil. According to Rattan., *et al*. (2005) [37] and Kowalik., *et al*. (2021) [28] sewage irrigation to crops resulted in alteration in the physical as well as chemical characteristics of soil and it leads to heavy metal build up. Sewage is rich in various chemical species and thus application of sewage to soil changes the physico-chemical characteristics (Gros., *et al*. 2006; Chithra and Jaya, 2005) [11,20]. Ladwani., *et al*. (2012) [29] reported that sewage application improves the soil's health status and crop growth while according to Hamza., *et al*. (2004) [21] and Tytla (2019) [48] sewage application deteriorated the soil quality and resulted in heavy metal build up.

Assessment of soil quality and health by assessing the changes in physico-chemical characteristics of soil are very common. Use of biological indicators to monitor soil quality is seems to be rather precise over phyisico-chemical methods. Because measurement of soil's biological activity is indirectly measuring the activity of micro-organisms prevailing there. Even a minor change in the natural characteristics of soil will clearly reflect on the activity, number and diversity of micro-organisms. The need for developing sensitive indicators of soil quality is growing since it promotes suitable soil management practices for long-term sustainability of terrestrial ecosystems. So, monitoring soil quality by means of biological indices can be of help for the management and sustainability of soils that received sewage application.

Vetiver grass (*Vetiveria zizanioides* (L.) Nash) is a tall (1–2 m), fast-growing, perennial tussock grass belongs to the family 'poaceae'. It has a long (3–4 m), massive and complex root system, which can penetrate to the deeper layers of the soil. Owing to its unique morphological, physiological and ecological characteristics such as its massive and deep root system, high tolerance to a wide range of adverse climatic and edaphic conditions, and fast growth, this grass has been used for many ecological restoration programmes such as soil and water conservation, rehabilitation, and remediation, wastewater treatment, its scented oils in the roots and as fodder for livestock (Britannica, 2020) [8].

Therefore, the major objective of this study is to elucidate the influence of sewage on the biological activity of soil under vetiver coverage by measuring the major microbial soil quality indicators.

Materials and Methods

Materials

For the study, Vetiver grass (*Vetiveria zizanioides* L. Nash) were collected from the medicinal garden in Kariavattom South Campus, University of Kerala in Thiruvananthapuram district. Plants were removed from the propagating soil and surface sterilized with distilled water to remove any adhering soil. Then the tops and roots of the vetiver sprouts were pruned to 10 cm and 5 cm respectively.

The homogenous raw sewage was collected from the sewage farm in Valiyathura, Thiruvananthapuram city, Kerala. Samples were brought to the laboratory and kept in the refrigerator to avoid the changes in physico-chemical characteristics. For heavy metal analysis 500 ml of samples was preserved by treating with HNO_3 so that the pH less than two. All the bio-chemicals and chemicals used for the estimations were of analytical grade. Heavy metals were analysed using Atomic Absorption Spectrophotometer (Perkin-Elmer PinAAcle 500, Singapore).

Experimental design

The healthy vetiver sprouts were transplanted to earthen pots (without drainage holes to prevent the loss of nutrients due to drainage) containing 3 kg of potting mixture (prepared by mixing the soil, sand and cow dung in the ratio 1:1:1) and allowed to grow under controlled conditions. The sprouts were irrigated with sufficient amount of tap water for 60 days for successful establishment and luxurious growth. Then the experimental plants were divided into control (C) and test groups (T). Sewage irrigation to the test groups were started on the 61st day of planting. Control plants were irrigated daily with 250 mL of tap water, and the test groups were treated on alternate days with 250 mL of sewage for the next 45 days. The total experimental duration was for 105 days. Soil samples were collected after the experimental period and the fresh soil samples were subjected to analysis of various biological parameters.

Methodology

The physico-chemical characteristics of sewage was determined following the standard procedures in APHA (2012) [5] and Trivedy and Goel (1998) [47].

Enumeration of soil microorganisms such as bacterial and fungal populations was done by serial dilution technique. Martin's agar plate method was used for fungal colonies and nutrient agar plates were used for bacterial colonies (Dubey and Maheswary, 2004) [15].

Total microbial Activity (FDA Hydrolysis) was determined following the method by Schniier and Rosswall (1982) [42]. Microbial Biomass Carbon (MBC) was determined by chloroform fumigation extraction method (Vance., *et al*. 1987) [49]. The active microbial biomass carbon (AMBC), Basal soil respiration (BSR) and microbial metabolic quotient (qCO_2) were determined following the procedures of Islam and Weil (2000) [25]. The microbial metabolic quotient (qCO_2) is the ratio of Basal Soil Respiration to Active Microbial Biomass Carbon. It is calculated as BSR per unit of AMBC.

Protease activity (μg Tyr (tyrosine equivalents) g^{-1} h^{-1}) was determined following the method as described by Speir and Ross (1978) [45]. Dehydrogenase activity (μg TPF g^{-1} h^{-1}) was determined by Triphenyl formazan method as described by Casida., *et al*. (1964) [9]. The activities of acid phosphatase (μg PNP g^{-1} h^{-1} at 30°C) and alkaline phosphatase (μg *P*NP g^{-1} h^{-1}) were determined following the procedure of Tabatabai and Bremner (1969) [46]. Activity of the enzyme peroxidise (POX) (μg H_2O_2 g^{-1} h^{-1}) were measured as described by Robertson., *et al*. (1999) [40]. Activity of invertase (μg Glu g^{-1} h^{-1} at 37°C) in the soils was determined following the procedure by Yao., *et al*. (2006) [52].

The statistical analysis of the data was done by UNIANOVA (Univariate Analysis of Variance) using SPSS 17 software.

Results

Physico-chemical analysis of sewage

Physico-chemical characteristics of sewage used for irrigation is given in table 1. Results show that the values obtained for pH, electrical conductivity (EC), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Ammonia-Nitrogen (NH_3-N) are above the permissible limits of discharge of wastewater prescribed by Environmental Protection Agency (EPA, 2002) [17]. EC and Total Phosphorus (TP) values are exceeding the maximum permissible concentrations prescribed by Food and Agricultural Organization (FAO, 1985) [18]. Total nitrogen (TN), Calcium (Ca), Magnesium (Mg) and total oil and grease (TOG) content recorded were also above the standard limit of Indian Environmental Standards (IES, 2006) [24]. Concentration of Potassium (K) in sewage was also above the maximum permissible concentrations of FAO (1985) [18]. Iron (Fe) content in the sewage was above the permissible limit set by National Environmental Quality Standards (NEQS, 1999) [33] for discharge into land or surface water bodies. It was also noted that concentration of the important toxic heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), zinc (Zn), mercury (Hg) and arsenic (As) in the sewage was also found several times above the maximum permissible limits set by IES, 2006 [24]. Therefore, the continuous discharge of untreated sewage to soil will certainly change the physico-chemical as well as biological characteristics of the natural soil.

Sl. No.	Parameter	Sewage	Permissible limit	Referred Agency/Organisation
1	pH	4.72 ± 0.11	5-9	EPA, 2002
2	EC (µS/cm)	4820 ± 3.14	250-300	FAO (1985)
3	BOD (mg L^{-1})	86.75 ± 1.2	40	EPA (2002)
4	COD (mg L^{-1})	2340 ± 1.5	120	EPA (2002)
5	NO_3-N (mg L^{-1})	1.57 ± 0.01	10	EPA (2002)
6	NO_2-N (mg L^{-1})	0.48 ± 0.001	1	EPA (2002)
7	NH_3-N (mg L^{-1})	31.41 ± 0.01	1	EPA (2002)
8	TN (mg L^{-1})	44.06 ± 0.5	25	IES, 2006)
9	IP (mg L^{-1})	4.27 ± 0.03	--	--
10	OP (mg L^{-1})	2.07 ± 0.01	--	--
11	TP (mg L^{-1})	7.48 ± 0.2	< 2	FAO (1985)
12	Silicates (mg L^{-1})	21.7 ± 0.2	--	--
13	SO_4^{2-} (mg L^{-1})	134.6 ± 0.4	250-400	IES (2006)
14	Cl^- (mg L^{-1})	142.6 ± 0.1	1500	EPA (2002)
15	Ca(as $CaCO_3$)(mg L^{-1})	214.7 ± 0.2	75	IES (2006)
16	Mg(as $CaCO_3$)(mg L^{-1})	95.3 ± 0.5	75	IES (2006)
17	Na (mg L^{-1})	125.44 ± 0.2	< 460	FAO (2002)
18	K (mg L^{-1})	95.6 ± 0.2	< 2	FAO (2002)
19	Iron (mg L^{-1})	3.7 ± 0.1	2	NEQS (1999)
20	Total Oil and Grease (TOG) (mg L^{-1})	261.7 ± 0.4	10	IES (2006)
21	Pb (mg L^{-1})	1.313 ± 0.001	0.1	IES (2006)
22	Cd (mg L^{-1})	2.681 ± 0.001	0.1	IES (2006)
23	Cr (mg L^{-1})	6.063 ± 0.001	0.3	IES (2006)
24	Zn (mg L^{-1})	11.493 ± 0.001	5.0	IES (2006)
25	Hg (mg L^{-1})	0.113 ± 0.001	0.01	IES (2006)
26	As (mg L^{-1})	1.567 ± 0.001	0.2	IES (2006)

Table 1: Physico-chemical characteristics of Sewage.

(Values are the mean ± SD of three replicates).

Changes in the soil characteristics

Changes in the characteristics of soils irrigated with sewage under vetiver coverage is shown in table 2. Results show that compared to the control soils, sewage irrigation significantly transformed all the soil quality characteristics under study.

After the experimental period pH, OC, concentration of heavy metals (Pb, Cd, Cr, Zn, Hg and As), enzymatic activities (protease, dehydrogenase, alkaline and acid phosphatase, peroxidase and invertase) and biological characteristics (MBC, AMBC, BSR, qCO_2, FDA analysis, bacterial and fungal population) of soil increased significantly.

Sl. No.	Parameter	Control Soil	Test Soil	Significance
1	pH	7.47 ± 0.02	7.58 ± 0.03	p = 0.05
2	OC (mg g^{-1})	5.3 ± 0.2	11.0 ± 0.1	p = 0.000
3	Pb (mg Kg^{-1})	BDL	1.003 ± 0.001	p = 0.000
4	Cd (mg Kg^{-1})	BDL	1.143 ± 0.001	p = 0.000
5	Cr (mg Kg^{-1})	BDL	2.999 ± 0.001	p = 0.000
6	Zn (mg Kg^{-1})	0.61 ± 0.001	4.700 ± 0.001	p = 0.000
7	Hg (mg Kg^{-1})	BDL	0.043 ± 0.001	p = 0.000
8	As (mg Kg^{-1})	BDL	1.103 ± 0.001	p = 0.000
9	Protease (µg Tyr g^{-1} h^{-1})	44.16 ± 0.1	52.18 ± 0.1	p = 0.000
10	Dehydrogenase (µg TPF g^{-1} h^{-1})	39.41 ± 0.1	44.19 ± 0.2	p = 0.000
11	Alkaline Phosphatase (µg p-NP g^{-1} h^{-1})	62.16 ± 0.1	66.64 ± 0.2	p = 0.000
12	Acid Phosphatase (µg p-NP g^{-1} h^{-1})	45.59 ± 0.2	51.55 ± 0.1	p = 0.000
13	Peroxidases (µg H_2O_2 g^{-1} h^{-1})	35.51 ± 0.2	49.47 ± 0.1	p = 0.000
14	Invertase (µg Glu g^{-1} h^{-1})	29.87 ± 0.1	32.03 ± 0.3	p = 0.000
15	MBC (µg g^{-1})	7640 ± 3	9470 ± 4	p = 0.000
16	AMBC (µg g^{-1})	520 ± 3	765 ± 3	p = 0.000
17	BSR (µg CO_2 g^{-1} day^{-1})	52.7 ± 1.2	65.6 ± 1.4	p = 0.000
18	qCO_2 (BSR/AMBC)	0.10 ± 0.001	0.09 ± 0.001	p = 0.000
19	FDA (µg FDA g^{-1} h^{-1})	41.5 ± 0.2	86.2 ± 0.4	p = 0.000
20	Bacteria (CFU g^{-1})	$111x10^{-5}$ ± 3	$136x10^{-5}$ ± 4	p = 0.000
21	Fungi (CFU g^{-1})	$21x10^{-3}$ ± 4	$35x10^{-3}$ ± 2	p = 0.000

Table 2: Changes in Soil characteristics after experimental period.

(Values are the mean ± SD of three replicates).

Discussion

Changes in the physico-chemical as well as heavy metal contents of soil

Result of the analysis of changes in the physico-chemical characteristics of soil shows that even if the pH of irrigated sewage was with an acidic range of 4.72, sewage irrigated soils showed a pH (7.47) more or less similar to control soil (7.58), after the experimental period. Both the test and control soils turned to slightly alkaline in nature.

Results showed that organic carbon content in the soil was increased after the experimental period. It may be due to the degradation of various biological organic substances present in sewage. Former studies also suggested that sewage irrigation build up organic carbon and organic matter in the exposed soil (Raychaudhuri., *et al*. 2014 and Shan., *et al*. 2021) [13,29].

Result shows there is an increase in the concentration heavy metals in the sewage irrigated soils. In control soil except Zn (0.61 mg K^{-1}), all the remaining five heavy metals (Pb, Cd, Cr, Hg and As) determined were below the detectable limit. Presence of Zn in control soil was may be contributed by natural origin and it is well within the prescribed limit (Adriano, 1984) [1]. But in test soils concentration of all heavy metals were increased to a significant level compared to control. The study revealed that with in the short time span, build-up of heavy metals was significant and a continuous application of sewage to soil will certainly build-up toxic heavy metals to a higher concentration, above the permissible limits.

Statistical analysis (UNIANOVA) also proved that changes in the concentration of organic carbon and heavy metals in the control and test soils were of highly significant (p = 0.000).

Changes in the enzymatic activities

Soil enzymes are the vital activators in life progressions and have a substantial role in maintaining the soil health and biochemical functions, which drives the overall process of organic matter decomposition in the soil system. A unique balance of chemical, physical and biological (including microbial enzymatic activities) constituents subsidise to sustaining soil health. The enzymatic activity in the soil is mainly of microbial origin. A better knowledge about soil enzymatic activities will potentially provide unique breaks for the integrated biological assessment of soils due to their decisive role in numerous vital soil biological activities, ease of measurements and quick response to changes in soil management practices (Bakshi and Varma, 2011) [7].

Soil enzyme activities were very much influenced by the application of sewage. Results (Table 2) show that due to sewage irrigation, activity of all the studied enzymes such as protease, dehydrogenase, alkaline and acid phosphatase, peroxidase and invertase were increased significantly ($p = 0.000$) compared to that of control.

Improvement in the activity of protease in the sewage irrigated soil represents high degree of mineralization of nitrogenous compounds by the specific micro-organisms. Dehydrogenases (DHA) are a group of intracellular enzymes present in active microorganism in the soil and are considered to be a good indicator of overall microbial activity (Dick, 1997; Phale., *et al*. 2019) [14,35] as it gives indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility and therefore it can be used as a measure of any disruption caused by toxic pollutants such as pesticides, trace elements, or management practices to the soil. So, an increase in DHA is directly correlated with increased number of micro-organisms in soil. Many studies also reported that measurement of DHA in soil is an indirect measurement of abundance of micro-organisms (Anjaneyulu*., et al.* 2011; Meena and Rao, 2021) [2,31].

The phosphatases are a vide group of enzymes, capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid and act as a good indicator of soil fertility. They have critical role in maintaining the phosphorus cycle and show correlation with P stress and plant growth (Bakshi and Varma, 2011) [7]. Peroxidase enzymes are antioxidant enzymes act upon hydrogen peroxide and release oxygen to the rhizospheric zone, where oxygen is naturally low and this will promote the growth and multiplication of aerobic micro-organisms in the soil by reducing the oxidative stress produced due to the presence of heavy metals (Zade*., et al.* 2010) [53].

Invertase is the enzyme that catalyses the hydrolysis of sucrose and yields glucose and fructose. These are also intracellular enzymes and a wide range of micro-organisms produce this enzyme by utilizing the sucrose as nutrient (Frankenberger and Johanson, 1983) [19].

Higher enzyme activities were noticed in the sewage treated soils. The stimulation of enzymes in the soil may be due to sewage irrigation and subsequent increase in suspended organic materials and nutrients in the soil. Sewage serves as a source of energy for microbes and enzymes. Several studies reported that enzyme activities of soil increased due to wastewater application (Jogan and Dasog, 2019; Antonious., *et al*. 2020) [3,27]. According to Han., *et al*. (2018) [22] use of reclaimed water for irrigation significantly increases the soil enzymatic activity.

The observed increase in the activity of all the selected enzymes in sewage treated soils could be attributed to the rhizospheric effect leading to a faster multiplication of the active microbial fauna involved in the production of specific enzymes triggered by secretions and excretions, and the enzymatic activities in soils shows the average activity of the active microbial population in that soil (Zade*., et al.* 2010) [53]. The thick massive roots of vetiver plants also triggered the multiplication of all these micro-organisms. Higher organic carbon support higher microbial population that leads to higher concentration of various enzymes such as dehydrogenase, phosphatases etc.

Heavy metals have a known negative impact on soil enzymes, as high concentration of heavy metals retards or decelerates the enzymatic activities (Zade., *et al*. 2010; Jaworska and Lemanowicz (2019) [26,53]. But in the present study, result was found just opposite to the known facts. Sewage used for irrigating the soil was rich in toxic heavy metals such as Pb, Cd, Cr, Zn, Hg and As (Table 1). Results also showed that due to sewage irrigation heavy metal concentrations were significantly increased in the soil (Table 2). Analysis of enzymatic activities in the soil samples showed that presence of high concentrations of heavy metals does not inhibit the soil enzymatic activities. Several previous studies reported that sewage is rich in various micro and macro nutrients, organic matter and inorganic compounds, and therefore sewage irrigation improved the growth of plants as well as micro-organisms in the receiving soil (Wolfyang and Dohler, 1995; Ladwani., *et al*. 2012) [29,51].

Previous studies show that vetiver plants (*Vetiveria zizanioides* L.Nash) are highly tolerant, fast growing, high biomass producing plants characterised with thick, massive and wide spreading network of root system, which harbours numerous indigenous population of microbial fauna in the rhizosphere soil (Dhanya and Jaya, 2010; Melato., *et al*. 2016) [13,32]. Therefore in the present study the nutrient rich sewage and rhizospheric activity was found beneficial to the vetiver plants, as they grown luxuriously by absorbing sufficient quantities of nutrients. Also, the massive root system of vetiver has stimulated the growth and multiplication of highly indigenous micro-organisms in the soil and there by reduced the negative impact of heavy metals on the enzymatic activities of the test soils.

Changes in the biological characteristics

Measurement of enzymatic activity and or microbial biomass in the soil alone does not provide information on microbial activity. Some measure of microbial activities such as microbial biomass carbon (MBC), active microbial biomass carbon (AMBC), basal soil respiration (BSR), carbon quotient (qCO_2), Fluorescien Diacetae (FDA) hydrolysis etc. also required for the exact assessment (Sparling and Ross, 1993) [44].

Soil microbial biomass plays a key role in nutrient cycling and ecosystem sustainability and has been established to be perceptive to increased toxicity and stress due to toxic heavy metals or other chemicals in soils (Elbl., *et al*. 2019) [16] and therefore the estimation of microbial biomass is highly beneficial in understanding the changes in soil biological properties. The present study reported that both the microbial biomass carbon (MBC) and active microbial biomass carbon (AMBC) in sewage irrigated soils was significantly high ($p = 0.000$) in comparison to the unpolluted control soil. MBC is the direct measurement of total quantity of microbial cells in the soil while AMBC is the direct measurement of live microbial biomass in the soil.

Result confirmed that sewage irrigation to the vetiver cultivated soil increased the microbial population to a significant level ($p = 0.000$). Microbial biomass is an agent of biodegradation of organic components in soil, by which dissolved organic matter is decomposed and organic pollutants are removed from the soil and the quantity of microbial biomass stands substantial fraction of the labile organic C pool in soils and it has direct relation with nutrient cycling of soil organic matter. Quantification of microbial biomass is used as an important susceptible indicator, which can function as a pool of macro nutrients or be catalyzed during the disintegration of organic matter and therefore as a source and sink of available nutrients, it plays a critical role in nutrient transformation (Masto., *et al*. 2011) [32].

Basal soil respiration (BSR) is commonly considered to designate the total carbon turnover in the soil and an estimate of total microbial activity, reflecting both the quantity and quality of the carbon sources. It is calculated by measuring the trapped CO_2 released during the respiration of micro-organisms. Higher soil respiration denotes a higher soil microbial activity, accompanied with rapid decomposition of organic residues that makes nutrients available for the consequent stimulation of heterotrophic microorganisms. Result shows that the BSR of the sewage treated soil was also higher than the untreated control soil and it again affirms increased microbial population in the test soil ($p = 0.000$).

The microbial metabolic quotient (respiration-to-biomass ratio, qCO_2), is the ratio of basal soil respiration to active microbial biomass (BSR: AMBC), is inversely related to the efficiency with which the microbial biomass uses the indigenous substrates (Masto., *et al*. 2011; Novak., *et al*. 2017) [30,34]. It is increasingly being used as an index of ecosystem development and disturbance (Wardle and Ghani, 1995) [52]. That is a lower qCO_2 showed lower soil chemical stress to microorganisms, more fruitful C utilization efficiency, less energy demand in microbial biomass maintenance and better soil quality (Cheng., *et al*. 2013) [10]. The lower qCO_2 in the experimental soil compared to control soil is again ascertained the healthy soil ecosystem of the sewage irrigated soil. Result show that there are significant changes in qCO_2 ($p = 0.000$).

Measurement of total microbial activity (FDA Hydrolysis) is used to evaluates the overall microbial activity including the activity of soil enzymes such as proteases, lipases etc. and is used as an index of microbial stress during pollution (Aparna., *et al*. 2010) [4]. The FDA activity symbolizes an estimation of total microbial activity, showing the global hydrolytic capacity of the soil for microbial decomposition of OM (Sanchez-Monedero., *et al*. 2008) [41]. Increased FDA hydrolysis in the present study therefore indicates an increased activity of the microorganisms due to OM contribution, macro and micronutrients, and pH increment by the sewage irrigation and a healthy population of active microbial fauna capable of degrading various organic substances in the soil. All these factors are necessary for microbial growth and soil activity.

Enumeration of bacterial and fungal population in the control and test soil samples also point out that sewage irrigated soil harbours more number of both bacteria and fungus than that in the control soil. Therefore, a higher number of microbial fauna and an associated increase in the enzymatic activities along with an increase in MBC, AMBC, BSR and FDA hydrolysis and decreased qCO_2 may be due to high organic matter content in the sewage irrigated soil. Irrigation with nutrient rich sewage is responsible for the growth of vetiver plants and associated micro-organisms in the rhizospheric zone supported with thick massive vetiver roots.

Conclusion

The study revealed that sewage irrigation in vetiver cultivated soils increased the heavy metal build-up but the values were within the permissible limit. This might be attributed by the increased microbial biomass contributed by the organic carbon rich sewage and the thick basement provided by the wide spreading root network of vetiver plants, which stimulated the growth, multiplication and activity of micro-organisms in the experimental, rhizospheric soil, by conquer the impact of heavy metals. Therefore, evaluation of biological properties (microbial and enzymatic activities) of sewage irrigated soils under vetiver coverage revealed that sewage irrigation does not have harmful effects on vetiver cultivated soils. The study also confirmed that evaluation of biological properties of soil is precise over physico-chemical examination of soils, to check the soil health status due to changes in the management practices.

Acknowledgements

Authors acknowledge the financial support granted for this work by the Kerala State Council for Science Technology and Environment (KSCSTE), Thiruvananthapuram, Kerala, India.

Bibliography

1. Adriano DC. "Trace Elements in the Environment". New York: Springer Verlag (1984).
2. Anjaneyulu E., *et al*. "Effect of Pig Iron Slag Particles on Soil Physico-Chemical, Biological and Enzyme Activities". *Iranica Journal of Energy and Environment* 2.2 (2011): 161-165.
3. Antonious GF., *et al*. "Monitoring soil enzymes activity before and after animal manure application". *Agriculture* 10.5 (2020): 166.
4. Aparna C., *et al*. "Evaluation of bioremediation effectiveness on sediments Contaminated with industrial wastes". *International Journal of Environmental Sciences* 1.4 (2010): 607-620.
5. APHA. "Standard Methods for the Examination of Water and Wastewater". 22nd ed., Washington, DC: APHA- AWWA- WEF.
6. Asano T., *et al*. "Water Reuse: Issues, Technologies, and Applications". New York, NY: McGraw Hill (2007).
7. Bakshi M and A Varma. "Soil enzyme: The State of Art". In: Soil Enzymology, Soil Biology, eds. G. Shukla and A. Varma, 22: 1-23. Berlin Heidelberg: Springer-Verlag (2011).
8. Britannica T. "Editors of Encyclopaedia". Vetiver, Encyclopedia Britannica (2020).
9. Casida LE., *et al*. "Soil elements or management-Dehydrogenase activity". *Soil Science* 98 (1964): 371-376.
10. Cheng F., *et al*. "Soil Microbial Biomass, Basal Respiration and Enzyme Activity of Main Forest Types in the Qinling Mountains". *PLoS ONE* 8.6 (2013): e67353.
11. Chithra VS and DS Jaya. "Phytoremediation of sewage by grass cultivation: A case study of Valiathura Sewage Farm in Thiruvananthapuram District, Kerala". *Asian Journal of Microbiology, Biotechnology and Environmental Sciences* 7.4 (2005): 693-698.
12. CPCB. "National Inventory of Sewage Treatment Plants". Central Pollution Control Board, Parivesh Bhawan, East Arjun Nagar, Delhi (2021): 183.
13. Dhanya G and DS Jaya. "Physio-biochemical changes in vetiver grass (Vetiveria zizanioides L.Nash) used for the phytoremediation of soils contaminated with different waste waters". Unpublished M.Phil Dissertation, Department of Environmental Sciences, University of Kerala (2010): 154.

14. Dick RP. "Soil enzyme activities as integrative indicators of soil health". In: Biological indicators of Soil Health, eds. C. E. Pankhurst, B. M. Doube and V. V. S. R. Gupta, 121-156. Wellingford: CAB International (1997). doi: 10.1080/15226514.2015.1115963.

15. Dubey R C and D K Maheshwari. "Practical Microbiology". Ist Edition. Ahmedabad, Gujarat, India: S. Chand Publications (2004).

16. Elbl J., *et al*. "Response of microbial activities in soil to various organic and mineral amendments as an indicator of soil quality". *Agronomy* 9.9 (2019): 485-488.

17. EPA. "The Environment Protection Act- 2002". Standards for Effluent Discharge Regulations, General Notice. No. 44 of 2003 (2002).

18. FAO. "Water quality for agriculture". In: Irrigation and Drainage Paper No. 21 (Rev. 1). eds. Ayers, R.S. and D. W. Westcot. 1-174. Food and Agricultural Organization, Rome: UNESCO Publication (1985).

19. Frankerberger WT and J B Johanson. "Method of measuring invertase Activity in soils". *Plant and Soil* 74 (1983): 301-311.

20. Gros R., *et al*. "Soil physico-chemical changes following application of municipal solid waste leachates to grasslands". *Water, Air, and Soil Pollution* 169 (2006): 81-100.

21. Hamza S., *et al*. "Influence of soil physico-chemical properties on productivity of black pepper (Piper nigrum L.)". *Journal of Spices and Aromatic Crops* 13.1 (2004): 6-9.

22. Han Y., *et al*. "Effects of different levels of irrigation with reclaimed water on soil enzyme activity and distribution of thermotolerant coliforms". *Huan jing ke xue= Huanjing kexue* 39.9 (2018): 4366-4374.

23. Hussain I., *et al*. "Wastewater use in agriculture: Review of impacts and methodological issues in valuing impacts". Working Paper 37. Colombo, Sri Lanka: International Water Management Institute (2002).

24. IES. "Indian Environmental Standards". In: Hand book of Indian Environmental Standards". ed. Raman, NS and S Devotte. National Environmental Engineering Research Institute, Nagpur (2006).

25. Islam K R and R R Weil. "Land use effects on soil quality in a tropical forest ecosystem of Bangladesh". *Agriculture, Ecosystem and Environment* 79 (200): 9-16.

26. Jaworska H and J Lemanowicz. "Heavy metal contents and enzymatic activity in soils exposed to the impact of road traffic". *Science Reports* 9 (2019): 19981.

27. Jogan H and G S Dasog. "Effect of Wastewaters on Soil Enzymes Activity". *International Journal of Current Microbiology and Applied Science* 8.4 (2019): 1080-1087.

28. Kowalik R., *et al*. "Risk Analysis of Heavy Metal Accumulation from Sewage Sludge of Selected Wastewater Treatment Plants in Poland". *Water* 13 (2021): 2070.

29. Ladwani K D., *et al*. "Impact of Domestic Wastewater Irrigation on Soil Properties and Crop Yield". *International Journal of Scientific and Research Publications* 2.10 (2012): 1-7.

30. Masto R E., *et al*. "Soil Biological and Biochemical Response to Cd Exposure". *Open Journal of Soil Science* 1.1 (2011): 8-15.

31. Meena A and K S Rao. "Assessment of soil microbial and enzyme activity in the rhizosphere zone under different land use / cover of a semiarid region, India". *Ecological Processes* 10.1 (2021): 1-12.

32. Melato F A., *et al*. "Adaptation and detoxification mechanisms of Vetiver grass (Chrysopogon zizanioides) growing on gold mine tailings". *International Journal of Phytoremediation* 18.5 (2016): 509-20.

33. NEQS. "National Environmental Quality Standards Regulations, 1999, National Environmental Management Act-1994 (Act No. 13 of 1994), Environmental Quality Standards Board, National Environment Agency, Banjul, The Gambia" (1999).

34. Novak E., *et al*. "Chemical and microbiological attributes under different soil cover". *Cerne* 23 (2017): 19-30.

35. Phale S., *et al*. "Microbial degradation of xenobiotics like aromatic pollutants from the terrestrial environments". In: Pharmaceuticals and personal care products: waste management and treatment technology, 259-278. Butterworth-Heinemann (2019).

36. Rai S., *et al*. "Comparative study of some physicochemical parameters of soil irrigated with sewage and canal water of Dehradun city, India". *Archives of Applied Science Research* 3.2 (2011): 318-325.

37. Rattan R K., *et al*. "Heavy metal contamination through sewage irrigation in Per-urban areas of National Capital territory of Delhi". Technical Bulletin, Division of Soil Science and Agriculture Chemistry. Indian Agricultural Research Institute, New Delhi (2005): 1-51.

38. Raychaudhuri R., *et al*. "Impact of urban wastewater irrigation on soil and crop". Research Bulletin No. 64. Directorate of Water Management (Indian Council of Agricultural Research) Bhubaneswar - 751023, Odisha (2014).

39. Reddy G R and K J Rao. "Impact of sewage irrigation on macronutrient status of soils". *Andhra Agricultural Journal* 47.3-4 (2000): 218-223.

40. Robertson G P., *et al*. "Standard Soil Methods for Long Term Ecological Research". Oxford: Oxford University Press (1999).

41. Sanchez-Monedero M., *et al*. "Fluorescein diacetate hydrolysis, respiration and microbial biomass in freshly amended soils". *Biology and Fertility of Soils* 44 (2008): 885-890.

42. Schniirer J and T Rosswall. "Fluorescein diaceate hydrolysis as a measure of total microbial activity in soil and litter". *Applied Environmental Microbiology* 43 (1982): 1256-1261.

43. Shan Y., *et al*. "Sewage sludge application enhances soil properties and rice growth in a salt-affected mudflat soil". *Scientific Reports* 2 (2021): 1402.

44. Sparling G P and D J Ross. "Biochemical methods to estimate soil microbial biomass: current developments and applications". In: Soil organic matter dynamics and sustainability of tropical agriculture. ed. Mulangoy, K. and R. Merckx. 21-37. Chichester: Wiley (1993).

45. Speir T W and D J Ross. "Soil phosphatase and Ssulphatase". In: Soil Enzymes, ed. Burns, R. G. 197-250. New York: Academic Press (1978).

46. Tabatabai MA., *et al*. "Use of p-nitrophenyl phosphate for assay of soil phosphatase". *Soil Biology and Biochemistry* 1 (1969): 301-307.

47. Trivedy PK and P K Goel. "Practical methods in Ecology and Environmental Science". India: Environmedia Publications (1998).

48. Tytla M. "Assessment of heavy metal pollution and potential ecological risk in sewage sludge from municipal wastewater treatment plant located in the most industrialized region in Poland—A case study". *International Journal of Environmental Research and Public Health* 16.13 (2019): 2430.

49. Vance ED., *et al*. "An extraction method for measuring soil microbial biomass Carbon". *Soil Biology and Biochemistry* 19 (1987): 703-707.

50. Wardle D A and A Ghani. "A critique of the microbial metabolic quotient (qCO2) as a bioindicator of disturbance and ecosystem development". *Soil Biology and Biochemistry* 27 (1995): 1601-1610.

51. Wolfyang W and H Dohler. "Heavy metals in Agriculture". *Journal of Soil Science and Plant Nutrition* 17 (1995): 195-198.

52. Yao H., *et al*. "Microbial bio-mass and community structure in a sequence of soils with increasing fertility and changing land use". *Microbial Ecology* 40 (2000): 223-237.

53. Zade S P., *et al*. "Soil enzyme: concepts and relevance in soil health and productivity". *An Asian Journal of Soil Science* 5.1 (2010): 225-235.