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RABBIT'S HEALTH AND ENVIRONMENTAL IMPACTS OF EXPLOITATION TREATED BIODEGRADABLE POLLUTION WATER BY JOHKASOU SYSTEM

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ABSTRACT

This paper is considered as a part of a multi-disciplinary analysis "Application of Decentralized On-Site Water Treatment System in Egypt for Use in Agriculture and Producing Safe Animal Proteins". The project aimed to investigate the environmental impact of implementing sewage water before and after treatment using the effluent of the on-site decentralized Japanese' Johkasou system. Blood chemistry of liver and kidney enzymes revealed significant increase in the fourth week in comparison with groups of treated and tap water. On the other hand, ALT and AST levels have no statistical differences between treated and tap water reared animals. The progressive pathological lesions were noted in 100% and 70% untreated water groups.

The benefit of Johkasou system model CE10 in treatment of sewage water and suitability for rearing of animals is demonstrated.

Keywords: Sewage water; Johkasou system; Pathology; Rabbit's health.

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INTRODUCTION

Sewage pollution is considered one amongst the foremost pollution issues that threaten human health at the international level. Egypt like several alternative countries is lacking sensible quality water resources, and pressures on these restricted resources are caused thanks to the improper disposal of excretion. Pollution will adversely influence the water resources. Major harmful water pollutants embody organic matter and malady inflicting organisms from waste discharges.[1]

Water is crucial to human and animal life. Water is vital to facilitate numerous functions of the body like digestion, metabolism, and elimination of wastes as mentioned in Ref. 2.

The contaminants in sewage water are classified into four categories: suspended solids, organic matter (Chemical oxygen demand or biochemical oxygen demand), nutrients (nitrogen and phosphorus), and heavy metals, which are the substances to be removed by conventional and/or advanced treatments for the purposes of discharge or treated water reuse.[3] Decentralized sewage water management, frequently consisting of aging and poorly maintained septic systems, is often encountered in rural areas, less densely populated regions, and municipal fringe developments where centralized wastewater collection infrastructure was unaffordable or unfeasible at the time of development. The decentralization treatment technology and management advancements have improved performance to the point where decentralized systems can be considered as permanent solutions to sewage water management. Johkasou system is a unique technology of domestic wastewater treatment.[4]

However, one of the biggest problems faced by mankind today is the mediocre quality of water in many parts of the world. There are many diseases caused by water pollution. These include gastrointestinal diseases, reproductive problems, neurological disorders, and even cancer. Babies, young children, elderly individuals, and pregnant women, especially those who have weakened immune systems, are more susceptible to illnesses from various water contaminants.[5]

Rabbit's production plays a considerable role in solving the problem of meat shortage in Egypt. However, the foremost obvious limitation to rabbit production is the availability of fresh, healthy and sensible quality water, that cause the impairment of production and feed potency as mentioned in the literature.[6,7]

The animal waste represents a lesser risk to human health than human faeces because of the "species barrier" and especially the species-specificity of most viruses. This assumption has had important consequences for the selection and prioritization of remedial interventions.[8]

For drinking water, the shift to preventive management is more advanced, with detectable changes in regulation and/or practice since the publication of the third edition of WHO's Guidelines for Drinking-water Quality which recommended a "Framework for Drinking-water Safety" and associated "Water Safety Plans".[9,10] However, problems remain of inadequate understanding of zoonotic risks and the inadequacy of fecal bacteria as indicators of risk. These routes of human exposure (recreational water use, drinking-water consumption and food products grown with animal excreta inputs) have the potential to transmit a range of hazards including pathogens (micro-organisms capable of causing disease in humans) and toxic chemicals, including heavy metals and pharmaceuticals, including drugs, antibiotics and their residues. These findings were observed in the literature.[11,12]

The aim of this work was to investigate the efficiency of sewage water with different Biochemical Oxygen Demand (BOD) levels on rabbit's health from biochemical analysis and pathological aspect in comparison with tap and treated water by the Johkasou system.

MATERIAL AND METHODS

Source of Sewage Water

During the experimental period, the sewage water was installed from toilets with holding tank systems and prepared with three different BOD levels through dilution of raw toilets sewage water by tap water (100% untreated raw sewage water, 70% untreated, 30% untreated and raw toilets sewage water treated by Johkasou system at the Faculty of Science — Cairo University. Johkasou systems designed for removing BOD, and nitrogen from sewage water.[13]

A small-scale Johkasou system designed for a single-family household of 5–10 equivalent persons was used in this study. It consisted of a capsule shaped tank made from plastic materials and is usually installed underground. Existing medium- and large-scale Johkaso systems are considered standard systems for treating sewage wastewater generated by up to 50 equivalent persons.

Treatment processes for BOD removal by aerobic microorganisms in a biological treatment system were considered. The microorganisms consume organic matters for metabolism, synthesis and energy hence the organic matter in wastewater is reduced with lowering of BOD concentration. In addition, the treatment methods using these principles are divided broadly into two types: fixed biofilm process, in which the microorganisms attach on the inert media; activated sludge process, in which the microorganisms suspend in the aerated mixed tank.

Animals

Fifty native rabbits aging approximately four weeks old were purchased from Giza villages of Egypt. All animals were left for one week to acclimatize under our laboratory conditions and fed with pelleted food and tap water *ad libitum* in the animal facility at Faculty of Veterinary Medicine, Cairo University.

Experimental design:

Fifty rabbits were randomly assigned into five groups with each group consisting of 10 animals. Group 1 (G1) reared on 100% untreated water, group 2 (G2) reared on 70% untreated water, group 3 (G3) reared on 30% untreated water, group 4 (G4) reared on treated water by Johkasou system and group 5 (G5) reared on tap water.

Five animals from each group were sacrificed at fourth week and the remaining animals were scarified at the end of experiment at sixth week. Blood samples were collected for measurement of serum biochemical parameters. Tissue specimens from different organs were collected and fixed in 10% neutral buffered formalin solution for the histopathological examination.

Measuring of sewage, treated and tap water standard parameters:

To measure the sewage water quality and drinkability we applied different chemical, and biological assessments. Measurement of water quality items included color, odor, pH, phosphate, hardness, chloride, ammonia, nitrite, nitrate; chemical oxygen demand (COD) and BOD were determined according to the standard methods for the examination of tap water and sewage water.[14]

The chemical analysis of collected water samples, including total hardness, was estimated by using the EDTA titrimetric method. Chlorides (CL) were estimated by the Argentometric method. Ammonia (NH3) concentration was determined by using "phenate method". Nitrite (NO2) level was estimated by the colorimetric method. Sulphate (SO4) concentration was determined by the gravimetric methods with drying of residues. Phosphate was estimated by using stannous chloride. Hydrogen ion concentration (pH value) was determined by using a digital pH meter (Jenway 3510, England).

Assessment of liver and kidney parameters:

(a) Total blood proteins by Colorimetric method.[15]
(b) Alanine transaminase (ALT) and Alanine amino transferase (ALT) by Colorimetric method.[16]
(c) Uric acid and Creatinine by Colorimetric method.[17]
(d) Urea by Colorimetric method.[18]

Pathological examination:

The rabbits were sacrificed and examined for any gross lesions especially liver, kidneys and urinary bladder.

For histopathology, tissue specimens from liver, kidney and urinary bladder from all experimental animals were taken and fixed in 10% neutral buffered formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4–6 micron) and stained with haematoxylin and eosin (HE).[19] Then, tissue sections were examined, and photomicrograph taken by using Olympus light microscope.

Statistical analysis:

Data were presented as mean $\pm$ SE (standard error). Statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) followed by Least Significant Difference LSD test. Statistical significance was acceptable to a level of $p < 0.05$. Data analysis was accomplished using the Statistical Package for Social Sciences (SPSS) software program (version 20).

RESULTS AND DISCUSSION

Since 2010, both Cairo and Nagoya Universities, have cooperated in a strategic project with the aim to localize the Japanese JOHKASOU bioreactor (as Packaged On-site Aerated Wastewater Treatment Plant or PAWTP) in Egypt. In this regard, Nagoya University donated a Johkasou bioreactor, (model CE10, Fuji Clean) Figs. 1(a)–1(b) to be installed at the Center of Inter (multi)-disciplinary Studies at Cairo University, where a large scientific group from the faculties of Science, Agriculture and Veterinary Medicine has been working on-site.

This paper describes the health problems associated with waste water irrigation and the technical solutions which have been developed to make it an economically attractive option. Special emphasis is given to the control of animal disease associated with grazing on sewage water-irrigated pastures. The present work aimed to evaluate the effects of untreated sewage and

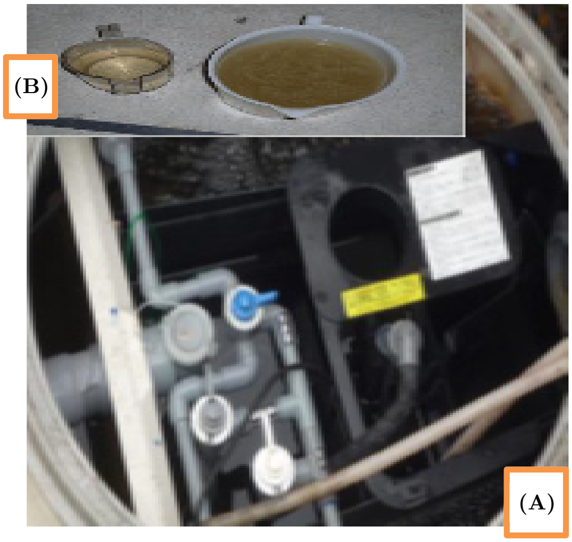

Fig. 1 Aerobic Chamber Sewage of Johkasou bioreactor (model CE10, Fuji Clean) **(A)** water before and **(B)** after treatment.

treated water by Johkasou system on liver, kidneys and urinary bladder of rabbits. The degrees of liver and kidney damages were evaluated biochemically and histopathologically.

Johkasou system is spreading and confidence in its performance is increasing greatly. Wastewater generated from household contains diverse types of organic pollutants represented by BOD concentration. There is also a fundamental problem of eutrophication of lakes and bays due to the contamination of nitrogen and phosphorous.

In the present study, water analysis was applied to compare between sewage and treated water by the Johkasou system based mainly on BOD, COD, Ammonia, Nitrate and Nitrite levels as shown in Table 1.

Elevated levels of Ammonia, Nitrate and Nitrite were recorded in sewage water groups. Therefore, a system that can remove nutrients such as nitrogen and phosphorous together with BOD is expected in the present era. Further research is going on for developing more efficient and easy-maintenance Johkasou systems. Future demand for Johkasou systems will be stronger with advanced treatment performance.[20]

Biochemical analysis of liver functions in this study depends upon alanine transaminases (ALT) as biomarkers to predict possible toxicity.[21] Generally, damage to liver cells will result in elevations of these transaminases in the serum.[22] Furthermore, measurement of enzymatic activities of ALT is of clinical and toxicological importance as changes in these activities are indicative of liver damage by toxicants or a diseased condition.[23] Significant changes of (ALT) in rabbits reared on 100%, 70% and 30% untreated water were observed during the experimental period. The specific activity of ALT was significantly lower ($p < 0.05$) in the control tap and treated water. There was an increase in the activities of liver AST in the fourth week that may have resulted from a leakage of these enzymes from the damaged liver to the serum.[24] On the other hand, aspartate amino transferase (AST) enzyme significantly decreased in the sixth week that indicated regeneration of hepatocytes and the animals become more adapted to source of water.

Also, activities of ALT and AST showed that there were significant differences among experimental groups. Total protein levels revealed no significant changes among experimental groups.

In addition, we compared the activities of liver enzymes with the normal physiological range.[21,22] Significant deviation in liver enzymes is presented in Table 2.

Table 1. Content Analysis of Distinct Types of Water Samples.

Parameters	**Water Samples**				
Source	Waste water (100%)	Waste water (70%)	Waste Treated water	Water (30%) Waste	Tap Water
Appearance	Turbid	Turbid	Turbid Clear		Clear
Color	Yellowish	Yellowish	Yellowish	Nil	Nil
Odor	Foul	Foul	Foul	Normal	Normal
pH	6.8	6.8	7	7.1	7.9
Phosphate (ppm)	28	18	10	3	4
Hardness (ppm)	170	170	170	170	280
Chloride (ppm)	340	190	140	100	130
Ammonia (ppm)	3	2	1	0.2	0.1
Nitrite (ppm)	4	4	2	0.02	0.01
Nitrate (ppm)	10	10	7	1	0.5
COD (ppm)	65	50	35	4.5	2.5
BOD	190	120	95	10	5

Table 2. Effect of Sewage, Treated and Tap Water on ALT (u/l), AST (u/l) and Total Protein (g/dl) Levels of Rabbits.

	ALT (IU/L)		AST (IU/L)		Total Protein (g/dl)	
Parameter Groups	**4 W**	**6 W**	**4 W**	**6 W**	**4 W**	**6 W**
G1	17.74 ± 2.2**	17.82 ± 0.53	75.21 ± 7.9**	7.21 ± 1.12	5.32 ± 0.31*	5.29 ±0.16
G2	14.79 ± 0.77*	17.22 ± 0.54**	72.9 ± 7.67*	6.25 ± 0.9*	4.62 ± 0.05	4.46 ± 0.2
G3	11.94 ± 0.98	14.87 ± 1.2[b]	36.03 ± 4.01	5.61 ± 0.13	4.58 ± 0.048	4.79 ± 0.24
G4	11.94 ± 0.78	11.88 ± 0.66	26.64 ± 1.1*	5.31 ± 0.03	4.75 ± 0.11	4.69 ± 0.29
G5	8.8 ± 1.2*	11.73 ± 0.82	18.28 ± 1.2	5.31 ± 0.03	3.74 ± 0.2	4.55 ± 0.03

Note: Data represented as mean ± S.E. ($n = 5$). The values within columns significantly different ($P \leq 0.05$).

The significant increase in biochemical parameters (AST and ALT) was manifested in pathological findings of examined animals that were reared in 100% and 70% untreated water. Liver showed distinct enlargement with focal dark areas scattered on the surface (Fig. 2). The cut section of examined liver was oozing blood in the presence of pen head size necrotic foci extended deep to hepatic parenchyma. These findings may be indicative of high rate of metabolism, absorption and elimination due to the presence of some toxic materials in the water such as ammonia, nitrate and some toxins produced by microorganism leading to multiple foci of coagulative necrosis and disorganization of hepatic cords with hyperplasia of kuffper cells and focal aggregation of mononuclear cells, mainly lymphocytes and macrophages as seen at the end of experiment (Fig. 3). These findings were similar to those observed by other authors.[25]

In the present study, the kidney function tests showed significant increase of urea and creatinine in all animals reared on untreated water in contrast to those reared on treated and control tap water as shown in Table 3.

On the other side, uric acid revealed no significant changes in comparison with the normal physiological range. This finding agreed with that recorded by Ref. 21. Furthermore, rabbits have a reduced capacity to concentrate urea and intestinal absorption, activity of the caecal flora, liver function, gastrointestinal hemorrhage, stress and hydration status can also influence blood urea nitrogen levels as recorded by Ref. 26.

Pathologically, kidneys revealed distension with stretched capsule in addition to dilatation of both renal pelvis and ureters by yellowish sticky urine. The kidney parenchyma showed varied degree of damage from one animal to another. The renal cortex revealed hypercellularity of glomerular tufts with accumulation of proteinous filtrate in Bowman's space. The renal tubules showed degeneration of its epithelial lining with mononuclear cells infiltration (Fig. 4). The renal medulla showed congestion of peritubular capillaries with sloughing of tubular epithelial lining forming intra-tubular cellular casts. These findings are in accordance with others[27,28] that studied the effect of uranyl nitrate ingestion in rats.

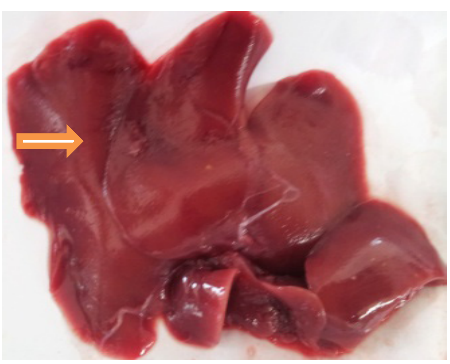

Fig. 2 Liver of groups 1 and 2 showing focal dark areas scattered on the serosal surface (arrow).

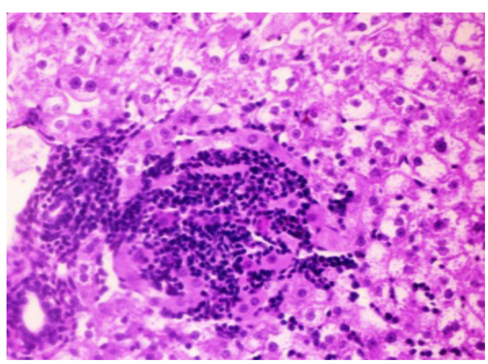

Fig. 3 Liver of groups 1 and 2 showing foci of coagulative necrosis with mononuclear cells aggregation (HE, x200).

Table 3. Effect of Sewage, Treated and Tap Water on Creatinine (mg/dl), Urea (mg/dl) and Uric Acid (mg/dl) Levels of Rabbits.

	Uric Acid (mg/dl)		Urea (mg/dl)		Creatinine (mg/dl)	
Group/Parameters	**4 W**	**6 W**	**4 W**	**6 W**	**4 W**	**6 W**
G1	1.64 ± 0.2	1.57 ± 0.19[a]	49.22 ± 5.1[b]	46.62 ± 2.3[a]	1.07 ± 0.03[b]	**0.60 ± 0.2[a]**
G2	1.11 ± 0.06[a]	1.57 ± 0.2[a]	34.3 ± 6.1[a]	43.99 ± 7.4[a]	0.65 ± 0.2[ab]	**0.56 ± 0.17[a]**
G3	1.1 ± 0.01[a]	1.32 ± 0.10[a]	31.25 ± 1.04[a]	42.05 ± 1.98[a]	0.61 ± 0.13[bc]	**0.57 ± 0.15[a]**
G4	1.08 ± 0.14[a]	1.22 ± 0.07[a]	31.01 ± 0.4[a]	40.06 ± 1.35[a]	0.42 ± 0.05[a]	**0.51 ± 0.16[a]**
G5	0.96 ± 0.05[a]	1.22 ± 0.04[a]	30.65 ± 1.6[a]	37.68 ± 4.4[a]	0.28 ± 0.09[a]	**0.40 ± 0.16[a]**

Note: Data represented as mean ± S.E ($n = 5$). The values within columns significantly different ($P \leq 0.05$).

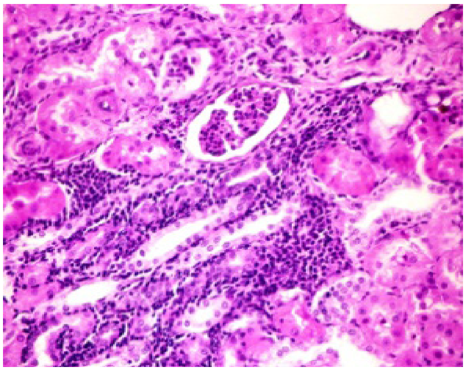

Fig. 4 Renal cortex of groups 1 and 2 showing tubular degeneration and leukocytic infiltration (HE, x200).

Over distension of urinary bladder with yellowish viscid granular urine was observed especially in group one (Fig. 5). The bladder mucosa showed degeneration and necrosis of its transitional epithelial lining with subepithelial oedema and congestion. Atrophy of urinary bladder folds was seen after six weeks. The over distention resulted in an immediate dysfunction in the contractility of the bladder that is not secondary to the reduction in intra-cellular ATP and is reversible within 1 week following over distention.[29]

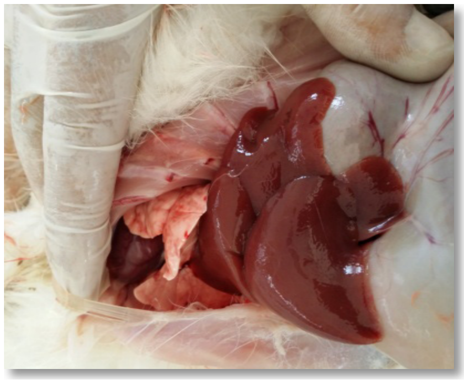

Fig. 6 Liver of groups 4 and 5 showing clear liver surfaces from any lesion.

On the other hand, in animals reared on treated water by Johkasou system and tap water, the post-mortem examination revealed normal anatomical and histological pictures of different organs included liver, kidneys and urinary bladder (Figs. 6 and 7).

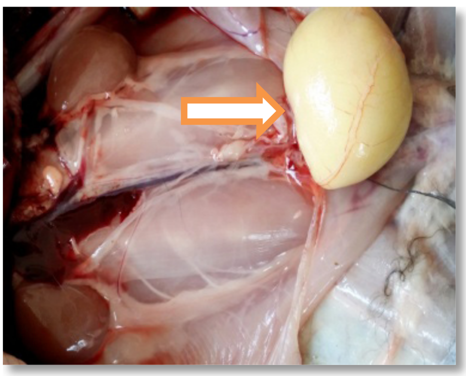

Fig. 5 Urinary bladder of groups 1–3 showing over distension with yellowish viscid granular urine (arrow).

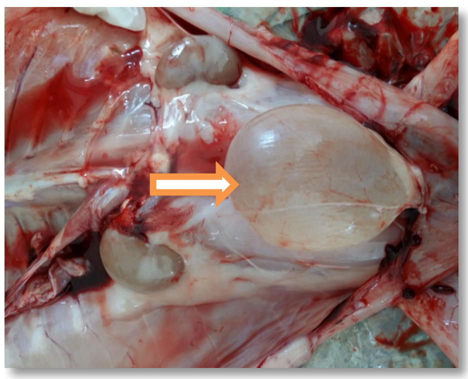

Fig. 7 Urinary bladder of groups 4 and 5 showing distension with clear urine (arrow).

In conclusion, the present paper demonstrates the benefit of Johkasou system model CE10 in the treatment of sewage water and suitability in the Egyptian environment. Rabbits reared on treated water showed healthier living conditions, as reflected by animal pathological findings in comparison with untreated water.

CONFLICT OF INTEREST STATEMENT

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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