



Original Research Article

Comparative Analysis of the Microbiological and Physicochemical Characteristics of Greywater Sources in Off-Campus Hostels at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

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ABSTRACT

Keywords

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The Comparative analysis of the Microbiological and physicochemical characteristics of greywater samples in Umuahia were carried out. The total heterotrophic plate count ranged from $8.1 \times 10^5 \pm 0.04$ cfu/mL – $1.11 \times 10^6 \pm 0.40$ cfu/mL, *Salmonella-Shigella* count ranged from $3.0 \times 10^2 \pm 0.02$ cfu/mL to $3.9 \times 10^2 \pm 0.10$ cfu/mL, *Escherichia coli* count ranged from $1.5 \times 10^4 \pm 0.02$ cfu/mL to $4.2 \times 10^4 \pm 0.05$ cfu/mL, *Enterobacter aerogenes* count ranged from $3.3 \times 10^2 \pm 0.05$ cfu/mL to $5.0 \times 10^2 \pm 0.04$ cfu/mL, the fungal count ranged from $2.0 \times 10^4 \pm 0.02$ cfu/mL to $3.9 \times 10^4 \pm 0.04$ cfu/mL while the coliform count ranged from 2100 ± 5.0 MPN/100mL to 11000 ± 25.0 MPN/100mL. The microorganisms isolated and their percentage occurrence were *Enterobacter aerogenes*, *Proteus* species, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* species, *Staphylococcus aureus*, *Rhizopus* species, *Penicillium* species, *Aspergillus* species and *Trichoderma* species. The mean values of the physicochemical parameters were also high. The results showed that the grey water samples were contaminated with known pathogenic microorganisms and should therefore be treated before reuse.

Introduction

Domestic wastewater generally is made up of black water and greywater. Black water is mainly wastewater from toilets, while grey water is non-industrial wastewater generated from domestic process such as washing of dishes, laundry and bathing (Lens *et al.*, 2001).

The environmental effects of the two wastewater types are not usually the same as they differ in the rate of decay of pollutants contained in them. Black water consists

largely of organic compounds that decompose slowly when placed in water while the decay of wastewater is faster owing to the presence of organics which are relative to the organics in black water, more readily available to microorganisms (Madungwe and Sakurungwa, 2007).

Greywater gets its name from its cloudy appearance and from its status as being neither fresh (white water) from groundwater or potable water nor heavily

polluted (black water). Essentially, any water other than toilet water draining from a household is grey water. Although this used water may contain grease, food particles, hair, and any number of other impurities, it may still be suitable for reuse (Agunwaba, 2001).

Greywater is domestic wastewater that is collected from dwelling units, commercial building, and institutions of the community. It may include process wastewater of industry (food, laundries, etc.) as well ground infiltrations and miscellaneous waste liquids. It is primarily spent water from building water supply to which has been added to the waste effluent of bathrooms, kitchens and laundry (Crook, 1991).

There is an increasing interest in the reuse of wastewater in many parts of the world, including both industrial and developing countries. One reason is water shortage caused by low amount of rainfall in combination with high evaporation or too large demands of freshwater from the population (Karina *et al.*, 2002).

In some countries, the driving force for the reuse of wastewater is environmental and economic considerations. The reuse will lower the total costs for wastewater handling since there will be a reduced load of water to the treatment plants.

Greywater is defined as wastewater without any input from toilets, which means that it corresponds to wastewater produced in bath tubs, showers, hand basins, laundry machines and kitchen sinks in household, offices, buildings, school etc.

Use of greywater for urinal and toilet flushing is one of the possibilities since the water that is used in many countries today is of drinking water quality. It has been estimated that 30% of the total household

water consumption could be saved by reusing grey water for flushing toilets (Karpiscak *et al.*, 1990)

The current water demand in large buildings revealed that not only greywater from bathrooms but also washing machine wastewater or storm water is needed to provide sufficient recycled water for non-potable uses (Surendran and Wheathey, 1998).

Outdoor applications for greywater could be irrigation of lawns on college campuses, athletic fields, cemeteries, parks and golf courses as well as in domestic gardens (Okun, 1997). Washing of vehicles and windows, fire protection, boiler feed water and concrete productions are examples of other suggested usages (Okun, 1997; Santala *et al.*, 1998).

The risk of spreading of disease due to exposure to microorganisms in the water will be a crucial point if the water is to be reused for example, toilet flushing or irrigation.

There is a risk that microorganisms in the water will be spread in the form of aerosols that are generated as the toilets are flushed (Albrechten, 1998; Christova *et al.*, 1996; Feachem *et al.*, 1983).

It is therefore necessary that when planning for the reuse of greywater to properly analyze the water with respect to physical, chemical and microbial qualities (Karina *et al.*, 2002).

The aim of the study was to determine the microbiological and physicochemical characteristics of greywater produced by students in Michael Okpara University of Agriculture, Umudike off-campus hostels in Umuahia, Abia State.

Materials and Methods

Sample collection

The grey water samples were collected early in the morning when cleaning in most lodges were at its peak. Three lodges were sampled during the visit. The lodges were Divine lodge, Presidential suites, and Glory lodge. Divine lodge provided samples from laundry activities; Presidential suites provided samples from shower activities while Glory lodge provided samples from kitchen activities. The samples were appropriately fixed in the field and transported on ice parked cooler to the laboratory where analysis commenced upon arrival.

Chemical Reagents

Chemical reagents used in the study were of analytical grade and were products of Hach Company, Colorado, USA; BDH Chemicals, Poole's, England and Sigma Chemical Company, St. Louis Missouri, USA. The microbiological media used were products of Oxoid and Difco Laboratories England. They were nutrient agar used for the estimation of total heterotrophic aerobic bacteria, purification and for stock culture; Sabouraud dextrose agar used for the isolation of fungi, *Salmonella-Shigella* agar for the isolation of *Salmonella* and *Shigella*, thiosulphate citrate bile salt sucrose agar for the isolation of *Vibrio cholerae*, MacConkey broth for coliform count and eosin methylene blue agar for the isolation of *Escherichia coli*

Enumeration of total heterotrophic bacteria and fungi

Samples of the stream water samples were serially diluted in ten folds. Total viable heterotrophic aerobic counts were determined using pour plate technique. Then

the molten nutrient agar, Sabouraud dextrose agar, *Salmonella-Shigella* agar, thiosulphate citrate bile sucrose agar and eosin methylene blue agar at 45°C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total heterotrophic bacteria and fungi, *Salmonella-Shigella*, *Vibrio cholerae* and *Escherichia coli* respectively. They were swirled to mix and colony counts were taken after incubating the plates at 30°C for 48h and preserved by sub culturing the bacterial isolates into nutrient agar slants which were used for biochemical tests.

Enumeration of coliforms

The coliforms were estimated using the Most Probable Number techniques (multiple tube fermentation technique) as described by Cheesebrough (2005).

Identification of bacterial and fungal isolates

Bacterial isolates were identified after studying the Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility, oxidase and catalase production; citrate utilization, oxidative/fermentation (O/F), utilization of glucose; indole and coagulase production, starch hydrolysis, sugar fermentation, methyl red-Voges-Proskaur reaction and urease production. The tests were performed according to the methods of (Ogbulie *et al.*, 1998; Fawole and Oso, 2001; Cheesebrough, 2005; Adeoye, 2007; Ochei and Kolhatkar, 2008). Microbial identification was performed using the keys provided in the Bergey's Manual of Determinative Bacteriology (1994).

Fungal isolates were examined macroscopically and microscopically using the needle mouth technique. Their

identification was performed according to the scheme of Barnett and Hunter (1972) and Larone (1986).

Physicochemical parameters

A number of physicochemical parameters of the grey water samples were determined. They included temperature, dissolved oxygen, pH, total dissolved solids, total suspended solids, turbidity and chloride. Others were nitrate, phosphate, sulphate, biochemical oxygen demand, chemical oxygen demand, calcium and sodium. The pH was measured *in-situ* using Hach pH meter (Model EC10); temperature and total dissolved solids were measured *in-situ* using Hach conductivity meter (Model CO150). The dissolved oxygen was also measured *in-situ* using Hach DO meter (Model DO175). Sulphate was determined using Barium chloride (Turbidimetric) method. Nitrate was determined using Cadmium reduction method while phosphate was measured using Ascorbic acid method. Biochemical oxygen demand was determined using Azide modification method. All analyses were in accordance with (APHA, 2005).

Results and Discussion

The results of the comparative analysis of the microbiological and physicochemical characteristics of greywater sources in off-campus hostels at Michael Okpara University of Agriculture, Umudike, Abia State are shown in tables 1–3.

Table 1 shows the mean counts of the microorganisms from the greywater samples. The total heterotrophic plate count ranged from $8.1 \times 10^5 \pm 0.04$ cfu/mL – $1.11 \times 10^6 \pm 0.40$ cfu/mL. The sample from laundry recorded the highest mean count of $1.11 \times 10^6 \pm 0.40$ cfu/mL while the sample from the kitchen sink recorded the lowest mean count of $8.1 \times 10^5 \pm 0.04$ cfu/mL. The

ANOVA, $P < 0.05$ showed that there was significant difference in the mean count among the different grey water samples. The *Salmonella-Shigella* mean count ranged from $3.0 \times 10^2 \pm 0.02$ cfu/mL to $3.9 \times 10^2 \pm 0.10$ cfu/mL. The laundry sample recorded the highest mean count $3.9 \times 10^2 \pm 0.10$ cfu/mL while kitchen sink sample recorded the lowest of 3.9×10^2 cfu/mL. The ANOVA, $P < 0.05$ there was significant difference the mean values among the grey water samples. The *Escherichia coli* count ranged from $1.5 \times 10^4 \pm 0.02$ cfu/mL to $4.2 \times 10^4 \pm 0.05$ cfu/mL with shower recording the lowest mean count of $1.5 \times 10^4 \pm 0.02$ cfu/mL while the kitchen sink recorded the highest count of $4.2 \times 10^4 \pm 0.05$ cfu/mL.

The ANOVA, $P < 0.05$ revealed that there significant difference in *Escherichia coli* mean count among the various grey water samples. The *Enterobacter aerogenes* count ranged from $3.3 \times 10^2 \pm 0.05$ cfu/mL to $5.0 \times 10^2 \pm 0.04$ cfu/mL. The laundry sample had the highest mean count $5.0 \times 10^2 \pm 0.04$ cfu/mL while the shower sample had the least mean count of $3.3 \times 10^2 \pm 0.05$ cfu/mL. The ANOVA, $P < 0.05$ revealed that there was significant difference in the mean count among the grey water samples. The fungal count ranged from $2.0 \times 10^4 \pm 0.02$ cfu/mL to $3.9 \times 10^4 \pm 0.04$ cfu/mL. The kitchen sink sample recorded the highest mean count of $3.9 \times 10^4 \pm 0.04$ cfu/mL while the shower sample recorded the least mean count of $2.0 \times 10^4 \pm 0.02$ cfu/mL. The ANOVA, $P < 0.05$ showed that there was significant difference in the fungal and coliform mean counts among the different sources of the grey water. The coliform count ranged from 2100 ± 5.0 MPN/100mL to 11000 ± 25.0 MPN/100mL. The highest mean count 11000 ± 25.0 MPN/100mL was recorded laundry sample and lowest mean count 2100 ± 5.0 MPN/100mL recorded kitchen sink sample.

Table 2 shows the microorganisms isolated from the grey water samples and their percentage occurrence. The bacteria isolated were *Enterobacter aerogenes* 23.8%, *Proteus species* 9.52%, *Escherichia coli*, 28.57%, *Pseudomonas aeruginosa* 19.05%, *Salmonella species*, 14.29%, *Staphylococcus aureus*, 4.79%. *Escherichia coli* had the highest percentage occurrence of 28.57% while *Staphylococcus aureus* had the lowest percentage occurrence of 4.79%. The fungi isolated were *Rhizopus species*, 20%, *Penicillium species*, 20% and *Trichoderma species*, 20%. *Aspergillus species* had the highest percentage occurrence of 40% and *Trichoderma species* had the least percentage occurrence of 10%.

Table 3 shows the mean value of the physicochemical parameters on the grey water samples. The mean values ranged as follows: pH, 5.95 ± 0.41 to 6.30 ± 0.42 ; Temperature, $26.6 \pm 0.5^\circ\text{C}$ to $27.2 \pm 1.6^\circ\text{C}$; turbidity, $41.0 \pm 1.0\text{NTU}$ – $45.0 \pm 3.0\text{NTU}$; Conductivity, $34.9 \pm 1.0\mu\text{S/cm}$ – $106.0 \pm 2.0\mu\text{S/cm}$; total dissolved solids, $100.0 \pm 3.0\text{mg/L}$ – $600.0 \pm 5.0\text{mg/L}$; total suspended solids, $265.0 \pm 4.0\text{mg/L}$ – $348.0 \pm 10.0\text{mg/L}$, dissolved oxygen, $10.35 \pm 0.83\text{mg/L}$ - $31.6 \pm 2.0\text{mg/L}$, biochemical oxygen demand, $3.1 \pm 0.04\text{mg/L}$ - $14.0 \pm 0.5\text{mg/L}$; chemical oxygen demand, $10.0 \pm 0.5\text{mg/L}$ – $20.0 \pm 1.0\text{mg/L}$; chloride, $60.0 \pm 2.0\text{mg/L}$ – $213.0 \pm 4.0\text{mg/L}$; Sulphate, $103.0 \pm 4.0\text{mg/L}$ – $163.0 \pm 10.0\text{mg/L}$; nitrate, $36.0 \pm 1.0\text{mg/L}$ – $70.0 \pm 5.0\text{mg/L}$, phosphate, $110.0 \pm 7.0\text{mg/L}$ – $200.0 \pm 15.0\text{mg/L}$; calcium, $40.10 \pm 2.0\text{mg/L}$ - $264.5 \pm 14.0\text{mg/L}$ and sodium, $57.2 \pm 2.8\text{mg/L}$ – $144.8 \pm 10.0\text{mg/L}$. The ANOVA, $P > 0.05$ showed that there was no significant difference in mean values for pH, temperature and turbidity. The ANOVA, $P < 0.05$ showed that there was significant difference in the mean values for the other parameters.

The result showed that the microbial counts were high and also the presence of pathogenic microorganisms in the greywater samples. This could be as a result of some households mixing children diapers with other clothes during cleaning, presence of organic carbon and solids and high concentrations of fats, oils and grease (Trujillo *et al.*, 1998; Wilderer, 2004). The presence of coliforms in the samples was seen as indicator of possible contamination with faecal materials. The presence of coliforms shows faecal contamination, signifying the presence of pathogens. The coliform measurements showed that the cleaning operations on most occasions increased the bacterial load of the greywater.

The factors that may contribute to the variation in the microbial counts were differences in hygiene conditions, variation in water economy that is some household recycle cleaning water more than others, variation in the period of in which the items being cleaned had been left soaking in the cleaning water. Food remains and possibly overnight soaking of used utensils may have contributed to high counts in dish greywater. The significant difference in the microbial counts in the greywater samples shows that the level of hygiene in the residential areas is different (Birks *et al.*, 2004).

The bacteria isolated from the greywater samples were *Enterobacter aerogenes*, *Proteus species*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella species* and *Staphylococcus aureus* while the fungi isolated were *Rhizopus species*, *Aspergillus species*, *Penicillium species* and *Trichoderma species*. The presence of these pathogenic microorganisms in greywater samples have been reported in earlier studies (Winward *et al.*, 2008). The Microbial contamination may pose a serious threat to health if greywater comes into contact with

humans during toilet flushing (Ericksson *et al.*, 2001, Burrows *et al.*, 1991). These microorganisms isolated from the greywater may release toxins in the effluent which can be very harmful. Fungal infection acquired through the environment causes allergies and only a few are transmissible from infected humans (Akani *et al.*, 2006; Oslen, 2007).

Escherichia coli is not specifically confined to the human intestine. It is also present in the faeces of many domestic animals and birds and can be source of contamination (Mullard, 1982; Wilson and Dick, 1990; Eze and Okpokwasili, 2008). The presence of *Staphylococcus aureus* may be from users and producers of greywater because it is a normal flora of the body and mucous membrane and most common aetiological agent of septic arthritis (Ellen and Sydney, 1990; Eze *et al.*, 2008). The consumer is at risk of acquiring food borne diseases. *Staphylococcus aureus* is the major cause of staphylococcal food poisoning.

The poisoning is characterized by diarrhea and vomiting (Singleton, 1995; Frazier and Westhoff, 2004; Eze *et al.*, 2008). *Pseudomonas aeruginosa* is an opportunistic pathogen commonly found in water, soil and vegetation. It also can be found in human and animal faeces. It rarely causes infection in healthy people but can colonize damaged systems, such as burn wounds and damaged eyes. *Salmonella* spp causes typhoid fever which is very common in the study areas.

The main reservoirs for human infection are poultry, cattle, sheep and pigs. *Salmonella* spp are common in animal faeces and wastes from slaughter houses and poultry processing plants (Brian, 1980). The occurrence of *Salmonella* could be due to the large number of animals and birds loitering in the study area. The positive

count of *Salmonella* in the greywater may be as result of an infected person washing partially cooked meat and shellfish or washing of uncooked meat in a bathroom washbasin and kitchen sinks (Birks *et al.*, 2004). Fish is a common meal in the study area and may also be the most likely source of *Salmonella* sp.

A wide variation in the physicochemical properties of greywater from the various sources can be attributed to the difference in the intensity of use of water before it qualifies to be discarded as well as the differences in the origin of dirt that the items being cleaned were exposed to. Other factors include the original quality of the water coming to the home, personal habits of the family members, the number of occupants, the age distribution of the members, their lifestyle, the type of chemical products used and the activities in the household (Bennett *et al.*, 2002).

The electrical conductivity from kitchen sink recorded highest value when compared with the other sources. The significant difference in the electrical conductivity values of the greywater from different sources may be attributed to the difference in the quantity of dissolved ions in the detergents used for the different cleaning operations (Waite *et al.*, 2006).

The pH of greywater depends on the pH of the water supply and the use of water for cleaning influences the pH of GW discharged (Christova-Boal *et al.*, 1996). The pH values were in line with the past research work which recommends a pH range of 5.0-8.0 for non potable interior and exterior wastewater reuse (Kelvin and Rinker, 1994). To avoid negative impacts on soil and plants when reused, raw untreated GW should have a pH in the range of 6.5–8.4 (FAO, 1997).

Table.1 The mean counts of microorganisms isolated from the grey water samples

	Cfu/mL						CC/MPN/10 0mL
	THBC	SSC	EC	EAC	FC		
KS	8.1×10 ⁶ ± 0.04	3.8×10 ² ± 0.02	3.1×10 ³ ± 0.03	3.3×10 ² ± 0.05	3.9×10 ⁴ ± 0.04		2100 ± 5.0
Showe r	9.8×10 ⁶ ± 0.07	3.0×10 ² ± 0.05	1.5×10 ³ ± 0.02	4.1×10 ² ± 0.04	2.0×10 ⁴ ± 0.24		4600 ± 10.0
Laund ry	1.11×10 ⁷ ± 0.40	3.9×10 ² ± 0.10	4.2×10 ³ ± 0.05	5.0×10 ² ± 0.04	3.0×10 ⁴ ± 0.07		11000 ± 25.0

Key: THBPC – Total Heterotrophic bacterial count, SSC – *Salmonella-Shigella* count, EC – *Escherichia coli* counts, EAC – *Enterobacter aerogenes* counts, FC – fungal counts, CC – Coliform counts, KS – Kitchen sink

Table.2 Microorganisms identified from grey water and the percentage occurrence

Bacteria	Number of isolates	% occurrence
Bacteria		
<i>Enterobacter aerogenes</i>	5	23.81
<i>Proteus species</i>	2	9.52
<i>Escherichia coli</i>	6	28.57
<i>Pseudomonas aeruginosa</i>	4	19.05
<i>Salmonella species</i>	3	14.29
<i>Staphylococcus aureus</i>	1	4.76
Fungi		
<i>Rhizopus species</i>	3	30
<i>Aspergillus species</i>	4	40
<i>Penicillium species</i>	2	20
<i>Trichoderma species</i>	1	10

Table.3 The mean values of the physicochemical parameters

Parameter	Laundry	Kitchen sink	Shower
pH	6.0 ± 0.41	6.20 ± 0.50	6.30 ± 0.42
Temperature (°C)	27.2 ± 1.6	26.6 ± 0.5	27.1 ± 1.0
Turbidity (NTU)	43.0 ± 2.0	45.0 ± 3.0	41.0 ± 1.0
Conductivity (µS/cm)	36.6 ± 0.90	106.0 ± 2.0	34.9 ± 1.0
Total dissolved solids (mg/Kg)	600.0 ± 5.0	110.0 ± 2.8	100.0 ± 3.0
Total suspended solids (mg/kg)	265.0 ± 4.0	348.0 ± 10.0	305.0 ± 6.0
Dissolved oxygen (mg/Kg)	31.6 ± 2.0	10.4 ± 0.83	17.8 ± 4.0
Biochemical oxygen demand (mg/Kg)	14.0 ± 0.5	3.1 ± 0.04	9.7 ± 0.10
Chemical oxygen demand (mg/Kg)	20.0 ± 1.0	10.0 ± 0.5	15.0 ± 0.8
Chloride (mg/Kg)	60.0 ± 2.0	213.0 ± 4.0	88.8 ± 2.8
Sulphate (mg/Kg)	163.0 ± 10.0	114.0 ± 8.0	103.0 ± 4.0
Nitrate (mg/kg)	70.0 ± 5.0	50.0 ± 2.0	36.0 ± 1.0
Phosphate (mg/Kg)	200.0 ± 15.0	170.0 ± 10.0	110.0 ± 7.0
Calcium (mg/Kg)	264.5 ± 14.0	48.10 ± 3.0	40.10 ± 2.0
Sodium (mg/Kg)	144.8 ± 10.0	102.0 ± 4.0	57.2 ± 2.8

The biochemical oxygen demand and chemical oxygen demand values of the greywater samples from the off campus hostels were much lower. This could be as a result of dilution of the samples due to volume of water used in the production. Kiplagat *et al.* (2011) suggested that the lower values were common during the wet season when water was relatively plentiful. Adequate dissolved oxygen is necessary for good water quality. Oxygen is a necessary element to all forms of life. Natural stream purification processes require adequate oxygen levels in order to provide for aerobic life forms. The dropping of dissolved oxygen level below 5.0 mg/L puts aquatic life under stress. The stress increases as the concentration of dissolved oxygen is lowered. Therefore greywater samples should not be stored for a long period to avoid the depletion of dissolved oxygen which may occur few hours after production. The depletion of dissolved oxygen in greywater may occur after 48 hours of production which allows the setting in of anaerobic process (Dixon *et al.*, 2000). The turbidity values were in line with other untreated greywater and the high value may be attributed to the levels of microorganisms in the greywater (Bitton, 1999; Casanova *et al.*, 2001). The mean values of nitrate, phosphate and sulphate were high. It has been shown that greywater is rich in total nitrogen and total phosphorus and has easily biodegradable organic content (Madungwe and Sakurungwa, 2007). Phosphates and nitrates are important ingredients to plant blooms and eutrophication of lakes and streams (Kiely, 1998). The relatively high sulphate values may be due to the application of sulphate containing detergents by the activities of the producers of greywater. High sulphate levels have been implicated in the composition of some locally formulated detergent (Odokuma and Okpokwasili, 1992; Okpokwasili and Olisa,

1991). The total suspended solids were on the high side which may be due to the activities that students in Off campus hostels engage themselves. The total dissolved solids of laundry were much higher compared to samples from kitchen sink and laundry. This may be attributed to the exposure of the clothes washed to environmental conditions that may lead to increase in total dissolved solids. Chloride levels were high in the samples and may be as a result of the type of detergents or soaps used. It has been shown that most commercially available bathroom/laundry products are currently manufactured using various types and quantities of sodium salts. Also household cleaning products are often sources of sodium, chloride and other salts (Wiel-Shafran *et al.*, 2006).

The study has shown that greywater samples contain pathogenic microorganisms and some physicochemical parameters signifying the pollution of the greywater. This demonstrates the risk that handlers of greywater are exposed to and therefore the need for the treatment of greywater before use and discharge into the environment.

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