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**OCURRENCE AND SEASONAL MONITORING OF HELMINTHES IN THE MAIN RIVERS AND LAKES OF GOIÂNIA, GOIÁS, BRAZIL, 2007****SÔNIA DE FÁTIMA OLIVEIRA SANTOS**

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**ABSTRACT:** Water constitutes one of the fundamental elements for human existence, which is demonstrated by its multiple uses, for public supply, industry, agriculture, livestock, recreation, and transportation. Nevertheless, contamination from various sources has been occurring for years impairing this resource and considerably increasing the risk of waterborne diseases caused mainly by bacteria, viruses, protozoa, and helminthes. Seasonal monitoring of helminthes is relevant to understand environmental contamination processes and provide information for decision-making measures to block the access of these parasites and other pathogens to man and animals. This study aimed at using parasitologic techniques to analyze the presence of helminthes and verify the water quality of the main bodies of water in Goiânia, the capital of the state of Goiás, Brazil. Samples were collected monthly from February 2006 to January 2007 in six pre-set points, in a total of 72 samples. Of these, 17 (23.61%) were positive for free-living larvae and 2 (2.77%) for *Ascaris* sp. and *Hymenolepis diminuta*. This study showed that the rivers and lakes of Goiânia are contaminated by eggs and/or larvae of helminthes.

**KEY WORDS:** Helminthes, water, waterborne diseases.

## OCORRÊNCIA E MONITORAÇÃO SAZONAL DE HELMINTOS NOS PRINCIPAIS RIOS E LAGOS DE GOIÂNIA, GOIÁS, BRASIL, 2007

**RESUMO:** A água constitui um dos elementos fundamentais para a existência humana, o que é demonstrado por seus múltiplos usos, para abastecimento público, industrial, agricultura, pecuária, recreação e transporte. No entanto, ao longo dos anos, vêm ocorrendo contaminações por várias fontes que têm provocado o comprometimento dos recursos hídricos e aumentado consideravelmente o risco de doenças de veiculação hídrica causadas principalmente por bactérias, vírus, protozoários e helmintos. O monitoramento sazonal de helmintos é relevante para o entendimento dos processos de contaminação ambiental e fornece informações que possibilitam a adoção de medidas para bloquear as vias de acesso destes parasitos e de outros patógenos ao homem e aos animais. Este estudo objetivou utilizar técnicas parasitológicas para a detecção de helmintos e verificar a qualidade das águas dos principais rios e lagos do município de Goiânia, Goiás, Brasil. As amostras foram coletadas mensalmente, de fevereiro de 2006 a janeiro de 2007 em seis pontos preestabelecidos, totalizando 72 amostras. Destas, 17 (23,61%) foram positivas para larvas de vida livre e 2 (2,77%) para *Ascaris* sp e *Hymenolepis diminuta*. Este estudo mostrou que rios e lagos de Goiânia estão contaminados por ovos e/ou larvas de helmintos.

**PALAVRAS-CHAVE:** Helmintos, água, veiculação hídrica.

### INTRODUCTION

A considerable decrease in water quality has been observed worldwide, caused by urban population growth at the end of the last century, which led to an expressive increase in the number of sources of pollution and, consequently, of enteric pathogenic microorganism population (Hunter et al., 1994). Due to their high clinical manifestation diversity, intestinal parasites represent a major problem in public health, mainly in countries where sanitation conditions are unsatisfactory. Among the factors contributing to the spread of parasites it is worth mentioning fecal contamination of soil and water (Ceccheto et al., 2007). Lack of treatment of residual waters brings danger to human health, since most waterborne diseases are related to unavailability of potable water and use of contaminated water (Hunter et al., 1994).

Among all pathogens, helminthes are enteric parasites often isolated from sanitary water that, together with other enteric pathogens, are transmitted through the fecal-oral route (Toze, 1999). Humans can be definitive, intermediate, paratenic, or accidental hosts (Slifko et al., 2000). Many helminth species are zoonotic, with high prevalence, and difficult to control, while some cause emergent or re-emergent zoonoses, with prevalence in both developing and developed countries (Nithiuthai et al., 2004).

Every year, 65,000 deaths caused by ancylostomide infections are recorded and 60,000 are associated to *A. lumbricoides* infections (Vercruysse et al., 2008). Infections caused by *Schistosoma* spp. represent a global public health problem that affects over 200 million people (McKerrow & Salter, 2002).

In 1978, the Public Health Department of the state of California, in the USA, published criteria establishing that the sewage treatment plant effluents used for irrigation of crops for human consumption should present fecal coliform count lower than 2.2 CFU.100 mL<sup>-1</sup> (California, 1978). Based on epidemiologic studies, in 1989 the World Health Organization published recommendations about microbiological standards, establishing that wastewater used in agriculture and aquaculture should present less than 1 egg of enteric nematode per 100 mL and fecal coliform count lower than 1,000 per 100 mL (WHO, 1989). Recently, the World Health Organization published a protocol on water and health with the purposes of promoting health and quality of life for all and showing the importance of this natural resource (WHO, 2006).

In Goiânia, the capital of the state of Goiás, Brazil, the João Leite river, affluent of the Meia Ponte river, is an important source of potable water for all the population. Nevertheless, it receives a huge discharge of urban, agricultural, and industrial effluents from Goiânia and the surrounding towns

and cities. Taking into consideration that the social economic situation and the cultural habits interfere in parasite transmission and prevalence (Bronfman et al., 1988), monitoring these pathogens in the environment has a social economic role, because the riverine population uses these supplies for several purposes and even to drink.

Goiânia also has innumerable lakes, such as Vaca Brava Park lake and Bosque dos Buritis lake, which offer a number of leisure activities for the population in general, but are not monitored for the potential presence of waterborne pathogens, including helminthes.

The aim of this study was to use parasitologic techniques to analyze the presence of helminthes and verify the water quality of the main rivers and lakes of Goiânia, Brazil.

## MATERIALS AND METHODS

### SPATIAL AND TEMPORAL SAMPLE DELIMITATION

Within a period of 12 months (from February 2006 to January 2007), water samples were collected monthly in sterilized 2-L polypropylene carboys from six points in the city of Goiânia: Meia Ponte river (n = 24), João Leite river (n = 24), Vaca Brava Park lake (n = 12), Bosque dos Buritis lake (n = 12), resulting in a total of 72 samples.

### MEIA PONTE RIVER

In this river, two sites were selected for sampling: the first, 1 km after the emission of wastewater treated by the municipal wastewater treatment plant of Goiânia, located at 16 37'40.94"S and 49 16'13.41"W (MP1), and the second, located at 16 38'22.39"S and 49 15'50.68"W (MP2).

### JOÃO LEITE RIVER

In this river, two sites were selected for sampling: one located at 16 37'40.18"S and 49 14'26.08"W (JL1), when this body of water reaches Goiânia, and the other located at 16 19'37.52"S and 49 13'24.53"W (JL2).

### VACA BRAVA PARK LAKE

This park encompasses an area of approximately 72.7 thousand m<sup>2</sup>, distributed among

green areas, walking and jogging tracks, sports courts, playground, exercise facilities and an artificial lake. The site selected for sampling is located at 16 42'31.18"S and 49 16'15.67"W (VB).

### BOSQUE DOS BURITIS LAKE

Bosque dos Buritis is an urban park encompassing an area of approximately 125 m<sup>2</sup> with three artificial lakes supplied by the Buriti stream. The site selected for sampling is located at 16 40'58.51"S and 49 15'38.35"W (BB).

### SAMPLING AND PROCESSING

We collected 5 gallons of water in clean polyethylene containers from one point in the center of the bodies of water approximately 20 cm under the surface and sent these samples within 2 h to the Genetics and Molecular Diagnostic Laboratory of the Universidade Federal de Goiás to undergo parasitologic analysis. Helminth larvae and eggs are commonly detected in water and sewage by microscopy (Gaspard and Schwartzbrod, 1995; WHO, 1989). Therefore, 500 mL of water were divided into two equal parts, sedimented for 1 hour, according to the method of Hoffman et al. (1934), standardized in APHA/AWWA/WEF (1998), and examined microscopically for helminth larvae and eggs by two different technologists. No quantitative analysis was performed on the positive samples.

### STATISTICAL ANALYSES

The results obtained in this study were digitalized in spreadsheets using Microsoft Office Excel 2007 software. Statistical analyses were performed using the chi-square test and the logistic regression analysis. Statistical significance level was set at  $p < 0.05$  using the Statistical Package for the Social Sciences (SPSS) version 10.0.

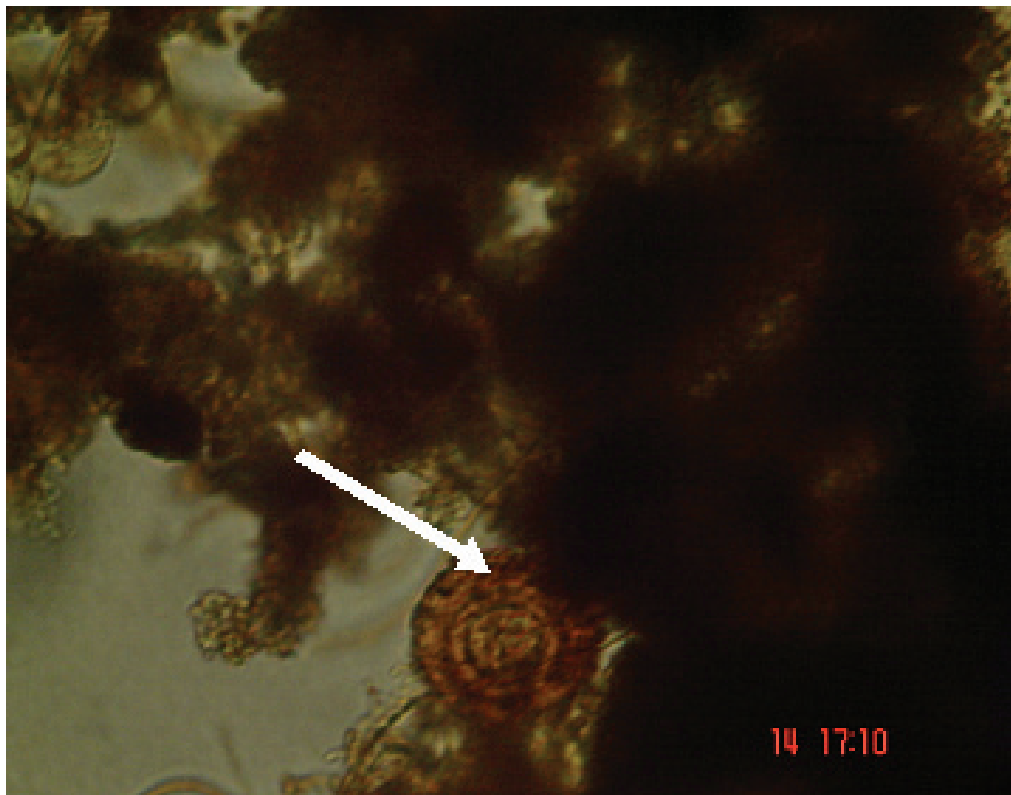
## RESULTS

Among all monitored months, only April and July did not show positivity for helminth eggs and/or larvae in any of the samplings (Table 1). In October, two helminth eggs and one *Ascaris* sp. egg were found at JL2 and in August, one *Hymenolepis diminuta* egg (Figure 1) was found at MP1.

**Table 1** – Occurrence of helminth eggs and/or larvae in the period of environmental monitoring, Goiânia, Goiás, Brazil.

Sampling site	Sampling periodicity – from February 2006 to January 2007											
	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan
MP1	+	+	-	+	+	-	-	+	-	-	+	-
MP2	-	-	-	-	-	-	-	-	+	+	+	-
JL1	-	-	-	-	-	-	-	-	+	-	-	+
JL2	-	-	-	+	+	-	+	+	-	-	+	-
VB	-	-	-	-	-	-	-	-	-	-	-	-
BB	-	-	-	-	-	-	+	-	-	-	-	-

(+) presence; (-) absence.



**Figure 1** - *Hymenolepis diminuta* egg found in water from the Meia Ponte river, Goiânia, Goiás, Brazil, 2006 (X 400).

Table 2 shows the general distribution of samples in the six sites according to the presence of helminthes from February 2006 to January 2007. The highest frequency of larvae positivity was 50% (6/12) at MP1 and all the other sampling sites presented results lower than that.

Dividing the samples according to two periods of the year, the dry (from April

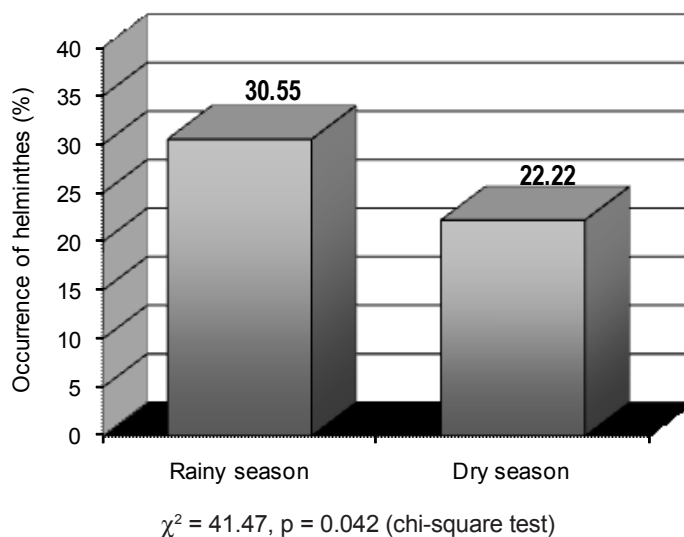
to September) and rainy (from October to March) seasons, it is possible to observe that the highest occurrence of helminthes happened in the rainy season, with 30.55% (11/36) positivity versus 22.22% (8/36) in the dry season ( $p = 0.042$ ) (Figure 2).

During the monitoring of the two lakes, just Bosque dos Buritis lake presented positivity for helminth occurrence, which

**Table 2** – General distribution of samples in the six sites according to the presence of helminthes, from February 2006 to January 2007, Goiânia, Goiás, Brazil.

Helminth	Sampling site												Total
	MP1		MP2		JL1		JL2		VB		BB		
	n	%	n	%	n	%	n	%	n	%	n	%	
Negative	5	41.7	9	75	10	83.3	6	50	12	100	11	91.7	53 (73.6%)
Helminth larvae	6	50	3	25	2	16.7	5	41.7	0	0	1	8.3	17 (23.6%)
<i>Ascaris</i> sp.	0	0	0	0	0	0	1	8.3	0	0	0	0	1 (1.4%)
<i>Hymenolepis diminuta</i>	1	8.3	0	0	0	0	0	0	0	0	0	0	1 (1.4%)
Total	12	100	12	100	12	100	12	100	12	100	12	100	72 (100%)

$\chi^2 = 37.221$ ,  $p = 0.171$ , chi-square test.



**Figure 2** – Occurrence of helminthes (eggs and larvae) in the dry and rainy seasons, from February 2006 to January 2007, Goiânia, Goiás, Brazil.

was very low since just one larva was found (August). In the two sites selected for sampling in the João Leite river, eight samples were positive: two at JL1 and six at JL2.

The highest frequency of helminthes (larvae and eggs), according to the month of sampling, was 15.78% (3/19), found in August, October, and December, compared to April and July, when no occurrence of this pathogen was recorded (Figure 3).

The average temperature in the period of helminth occurrence was 26.4°C, while in the period showing no occurrence of this pathogen, it was 25.5°C. The logistic regression analysis for temperature revealed  $p = 0.354$  and  $OR = 0.627$  (Table 3).

The average relative humidity in the period of helminth occurrence was 49.7%, whereas in the period showing no occurrence of this pathogen, it was 57.0%. The logistic regression analysis for relative humidity revealed  $p = 0.161$  and  $OR = 1.171$  (Table 3).

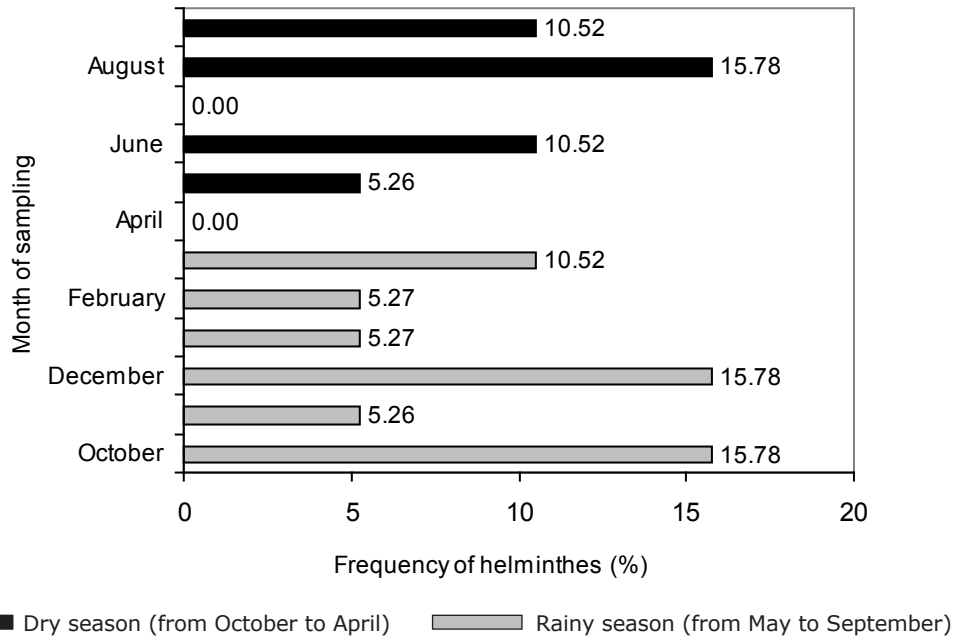
## DISCUSSION

Several studies have shown the importance of analyses to detect helminth eggs and larvae in water (Kozan et al., 2007; Moubarrad & Assobhei, 2007; Victorica & Galván, 2003). In Brazil, the Ministry of Health established the procedures and responsibilities related to

the control and surveillance of the quality of water for human consumption and its potability standards (Brasil, 2004). Although there are available data on waterborne diseases in Brazil and the regulations and tests used to guarantee the microbiological safety of drinking water are considered adequate, there is no integration of the governmental health plans and programs and the basic sanitation. The governmental health programs have been limited to merely corrective actions, not prioritizing prevention strategies and investments in basic sanitation, which could at least minimize the spread of enteropathogens and contamination of water supplies.

Nowadays, in Brazil as well as in other countries, it has become very common to use wastewater for crop irrigation purposes, thus enhancing the potential risk of enteric nematode disease transmission to humans, among which are *A. lumbricoides* and *T. trichiura* (Ayres et al., 1992), mainly due to the prolonged survival of these pathogens in the environment (Ghiglietti et al., 1995). The use of wastewater in agriculture poses a significant risk to human health mainly in tropical zones, where high humidity maximizes the prevalence of helminthes and other pathogens in the environment.

Nevertheless, low occurrence of helminthes was already expected for these lakes



**Figure 3** – Frequency of helminthes (eggs and larvae) in the bodies of water monitored, from February 2006 to January 2007, Goiânia, Goiás, Brazil.

**Table 3** – Mean and standard deviation of temperature and relative humidity according to the presence of helminthes in the bodies of water sampled from February 2006 to January 2007 in Goiânia, Goiás, Brazil (logistic regression analysis).

Helminthes	n	Mean	Standard deviation	p	OR
----- Temperature -----					
Negative	53	25.5	2.5		
Positive	19	26.4	2.4	0.354	0.627
----- Relative humidity -----					
Negative	53	57.0	16.2		
Positive	19	49.7	15.5	0.161	1.171

because there is no wastewater dumping into them. This does not remain true for the other two bodies of water sampled, constantly submitted to degradation processes due to anthropic action, such as agriculture, intensive livestock production and urbanization (Silva et al., 2010). Constant clandestine sewage discharge is evident, mainly in the Meia Ponte river, which presents the most severe problems.

JL1 is a site located before Goiânia and, consequently, it presents lower level of water pollution. As this creek crosses the city, it receives clandestine sewage discharges and

becomes more and more polluted, enhancing the chances of pathogenic microorganism occurrence. This is patent when analyzing the results found for JL2, located in the urban perimeter of Goiânia. This fact is highly worrying because in both sites (JL1 and JL2) we could observe the presence of people collecting water for human consumption, children and adolescents swimming and playing, and even adults fishing.

Among the ten positive samples collected in both sites of the Meia Ponte river, seven came from MP1, located 1 km after the emission

of wastewater treated in the municipal wastewater treatment plant of Goiânia (still being implemented), and three from MP2, located after Goiânia. Based on these results it can be inferred that the water at MP1 is more contaminated with helminthes than at MP2. One reason for this might be the low efficiency of the municipal wastewater treatment plant to remove these pathogens from water. On the other hand, the low positivity found at MP2 might be a consequence of the natural water depuration process as the river crosses Goiânia.

Chi-square test result ( $p = 0.171$ ) shows no significant differences in helminth (larvae and eggs) distribution according to the sampling site since the same proportion of occurrence could be observed among them.

It is important to emphasize that the World Health Organization suggests the limit of one egg of *A. lumbricoides* per liter of treated wastewater for crop irrigation purposes (WHO, 1989). However, along the Meia Ponte river, mainly near MP1 and MP2 sites, there are high population density areas. There the riverines, mostly low-income people, are not serviced by wastewater treatment and sewer collection systems. Therefore, they almost always have to collect raw water from the Meia Ponte river for various human purposes, especially to water vegetables, a common practice that was observed during the monitoring period in this body of water, where one egg of *A. lumbricoides* was found. Recent researches show the same reality in other parts of Brazil as well as in other countries all over the world, focusing on the prevalence and potential risks to human health after ingestion of raw vegetables contaminated with helminthes and other parasites (Kozan et al., 2007; Gupta et al., 2009; Silva et al., 2005). This can affect not only the riverines, but also any other people that consume the vegetables grown under these conditions.

Another interesting fact is the influence of precipitation on the sampled period. In Goiás, there are two well-defined seasons, a dry and a rainy one. The dry period lasts for 5 to 6 months, the highest rain volume is observed in spring-summer, and approximately 88% of the annual volume of precipitation is

registered from October to April. Goiânia has not gotten a hydrologic profile to establish precisely the beginning and the end of the dry and rainy seasons. For the purposes of this study, and based on the data registered by the Meteorology and Hydrology System of the state of Goiás, it was established that the rainy season in the region lasts from October to March and the dry season, from April to September.

Applying the chi-square test, a statistically significant difference was observed for the samplings in these two seasons ( $p = 0.042$ ). This finding is supported by recent researches in Spain showing increased prevalence of *Giardia* sp cysts and *Cryptosporidium* sp oocysts in the rainy season (Carmena et al., 2007).

Using the logistic regression analysis, both for temperature and relative humidity, no statistically significant results were found, demonstrating that these two parameters did not influence the occurrence of the monitored helminthes.

Although the analyses carried out in this research were qualitative, sample positivity indicates active contamination. The methodology applied to this study proved to be a practical low-cost tool. It can be used by laboratories to diagnose water parasites when they do not have the financial conditions to perform analyses using the membrane filter methods, which are expensive and difficult to purchase (APHA, 1992).

This is the first study in the state of Goiás to detect helminthes in this water bodies and it pointed to the existence of conditions that favor the occurrence of diarrhea episodes among the population of Goiânia, mainly children and elderly people, who do not have a very active immune system. Immediate intervention measures should be taken by decision makers, since the bodies of water sampled represent a real risk to the population that directly or indirectly has contact with them. The State must care for population well-being, a right ensured by the Brazilian Federal Constitution (Brasil, 1988).

## CONCLUSIONS

In this study it was possible to conclude that:



1. The rivers and lakes of Goiânia are contaminated by eggs of helminthes such as *Ascaris* sp. and *Hymenolepis diminuta* and larvae of helminthes;

2. The seasonal environmental monitoring revealed that the highest number of contaminated samples occurred in the rainy season;

3. The methodology used for the detection of eggs and/or larvae of helminthes is efficient, easy to process, and has low cost;

4. Clandestine sewage discharges and lack of basic sanitary conditions were evident, enhancing the chances of the population that lives near the courses of water to acquire waterborne diseases.

## RECOMMENDATIONS

There is the need to regularly biomonitor the quality of water of the rivers and lakes studied due to the imminent risk of increased frequency of cases of helminth infection as well as the possibility of occurrence of new pathological agents.

The residues generated by the municipal wastewater treatment plant of Goiânia must be rigidly controlled as to contamination since they can be used in agriculture as fertilizers.

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