

Influence of Polluted SY River on Child Growth and Sex Hormones*

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Abstract

Objective To investigate the influence of the polluted SY River on children's growth and sex hormones, and provide scientific data for assessment of the polluted status of the SY River.

Methods The study areas were selected randomly from the SY River Basin. Lead (Pb), mercury (Hg), arsenic (As), phthalates (DEP, DBP, DMP, DEHP), and bisphenol A (BPA) were measured both in the river water and in the drinking water. School children were selected by cluster sampling ($n=154$). Physical development indexes (height, weight, bust-circumference, and skinfold thickness) and sex hormones [testosterone (T) and estradiol (E2)] were measured for all the children.

Results The contents of Pb and Hg exceeded Class V standards of surface water quality in each section of the river and other indicators exceeded Class III. Compared to the control area, the concentrations of Pb, Hg, As, BPA, DEP, and DBP in the drinking water were significantly higher than in the polluted area ($P<0.05$). Children from the control area had significantly lower E2 and T than children from the polluted area ($P<0.05$). Among anthropometric results, only skinfold thickness had statistically significant difference between the two groups ($P<0.05$), while the other indexes showed no significant differences between the two groups ($P>0.05$).

Conclusion The drinking water has been polluted by the SY River and affected serum sex hormone levels of children living in the polluted area.

Key words: Water pollution; Children; Growth and development; Estradiol; Testosterone

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INTRODUCTION

The Huai River is the third largest river in China, which runs through Henan, Anhui, Shandong, and Jiangsu provinces. It has a total length of 1 000 km and covers an area of 270 000 km². About 150 million people live in the Huai River Basin. With population growth, economic development and rapid acceleration of urbanization since the 1980s, domestic sewage, industrial

effluents and agricultural pollutants have been poured into the river, making the water quality increasingly worse.

The SY River is located in the hinterland of Henan Province and is the largest tributary to the Huai River. With a total length of 418 km, it runs through the cities of Pingdingshan, Yuzhou, Xuchang, Luohe, and Zhoukou, and covers an area of 34 400 km². Various types of wastewater from 31 cities flow into the river without any sewage disposal.

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Some researches showed that the SY River had lost its self-purification capacity because of receiving so much municipal and agro-industrial wastewater^[1-3]. The SY River is one of the most polluted rivers in the Huai River Basin with water quality classified as Class V or worse^[2]. According to the monitoring data, a variety of harmful pollutants have been detected in the groundwater near the SY River at a depth of 20-50 m or deeper^[4]. Previous studies showed that water pollution could cause health damage such as increasing incidences of cancer^[5] and that the heavy metal contaminants in water could damage the body's nervous system and interfere with the endocrine system even at a low concentration^[6].

The effects of the Huai River pollution on health of local people are a major issue of social concern, which have been reported by many studies^[7-8]. According to one study, the incidence of malaria was connected with the distance from the river and 74% of malaria cases were inhabited within the extent of 60 m near the Huai River^[7]. Another study revealed that the pollution level of the Huai River water had a significant positive correlation with the incidence of gastric cancer, liver cancer, esophageal cancer, and other malignant tumors among local people^[8]. However, most of the observations have paid more attention to the adult population, and health researches on children and adolescents are rarely reported. As children are in a critical development period, especially those aged 8-13 years, they are more sensitive to environmental pollution than adults. The groundwater was one of the most important sources of drinking water to the local people, and there were no other sources of pollution in the two survey areas in our study. Therefore, the objective of this study was to evaluate the polluted status of the SY River and the drinking water and investigate the influence of water pollution on children's health, which would provide a scientific basis for selecting monitoring indicators.

MATERIALS AND METHODS

Location

The cross sectional study was conducted in S County which is the last county that the SY River runs through in Henan Province. The village located less than 2 km and the other one located more than 20 km away from the SY River Basin were randomly selected as a polluted and control area, respectively. The two villages were similar in the economic level,

demographic composition and living habits.

Subjects

Children aged 8 to 13 years old who were born and lived in the polluted and control areas were selected by cluster sampling. They were all the primary school students ($n=154$, 73 boys and 81 girls). 69 of them were from the polluted area (30 boys and 39 girls) and 85 from the control area (43 boys and 42 girls).

Sampling and Determination of Water Pollutants

The total length of the SY River is 32.5 km in S County. Three sampling sections were set up at S County. The upstream section (US) was set up at the place where the SY River just enters the county, the midstream section (MS) at the Huadian sluice gate and the downstream section (DS) at the place where the SY River runs out of the town. Three samples were collected from each section by quartering, and each water sample was measured three times. Drinking water samples were collected from five positions (east, south, west, north and central) of two villages, respectively, and three samples were collected from each position and each sample was measured two times.

The concentrations of pollutants including Pb, Hg, As, Bisphenol A (BPA) and dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and dioctyl phthalate (DEHP) were measured for both the river water and the drinking water. All indicators were tested in accordance with the monitoring and analytic methods for surface water and wastewater^[9].

Questionnaire

The questionnaire included children's birth and feeding patterns, birth weight, maternal education, passive smoking status, the number of days in a week for breakfast, exercise time, sleep time, the pesticide exposure of the mother during pregnancy etc.. Investigators were trained in advance and parents of students were visited by face to face interview.

Anthropometric Measurement

Participants' weight, height, bust circumference and skinfold thickness were measured with underwear only and without shoes. Body weight was

measured with a digital lithium weighing scale, height with a portable height and sitting height meter and bust circumference with a Gulick anthropometric tape. Skinfold thickness on triceps was obtained by using a Harpenden caliper.

Sex Hormone Analyses

The subjects were requested to fast for at least 10 hours before they came for the study. The fasting blood samples, totaling 5 ml for each subject, were drawn into vacuum tubes. Immediately upon collection, samples were stored on ice. At the end of each daily collection, samples were centrifuged to separate serum and stored at -20 °C until analyses. Testosterone (T) and estradiol (E2) were measured by an automated chemo-iluminescence analyzer. During the process of determination, a parallel sample were measured every 7 samples. At the end of the measurement of all samples, 20 samples were randomly selected and retested. Kit was provided by Autobio Diagnostics Co. Ltd. (Zhengzhou, China).

Statistical Analysis

The database was established by using Epidata 3.0 software (Epidata 3.0 for windows, Epidata Association Odense, Denmark) with data double entered into the database by different people. All data were analyzed with SPSS 12.0 software. Kolmogorov-Smirnov test and Levene test were used to inspect the normality and homogeneity of variance of all data, showing that all data were in line with normality and homogeneity of variance. The distribution of the basic situation of children such as birth and feeding patterns, birth weight,

maternal education, passive smoking status and so on was assessed by using the Chi-Square test. The levels of pollutants in river water samples were summarized with descriptive statistics. One-way analysis of variance (ANOVA) tests was used to compare mean differences in levels of pollutants of the three sections. The concentrations of pollutants in the drinking water and the indicators of physical development and sex hormone levels of children in the two areas were compared by using Independent-Sample T test. (significance level $\alpha=0.05$).

RESULTS

The Balance of Survey Subjects

Recovered valid questionnaires were analyzed, and the results showed that children in the two areas had no significant difference in the birth and feeding patterns, birth weight, maternal education, passive smoking, exercise time, sleep time etc. ($P>0.05$).

SY River Water Pollutant Levels

Compared with the standards of surface water quality^[9], the concentrations of Pb and Hg exceeded Class V in each section and other indicators exceeded Class III. In addition to As, there were no significant differences in the concentrations of all concerned substances among the three sections ($P>0.05$). Compared with the US and DS, the concentrations of As in MS were significantly lower ($P<0.05$), but there was no significant difference between the US and DS ($P>0.05$) (Table 1).

Table 1. Concentrations of 8 Pollutants in SY River Water ($\bar{x} \pm s$)

Indicators/Section	US (n=9)	MS (n=9)	DS (n=9)	F	P
Pb (μg/L)	52.100±8.921	70.467±12.888	64.800±7.879	2.587	0.155
As (μg/L)	50.138±5.563	33.447±5.564*	48.283±8.499	5.615	0.042
Hg (μg/L)	2.244±0.140	2.282±0.279	2.402±0.145	0.521	0.619
DMP (μg/L)	69.107±7.663	73.951±14.902	75.975±10.712	0.283	0.763
DEP (μg/L)	81.103±18.503	80.406±6.812	82.240±15.219	0.012	0.988
DBP (μg/L)	11.613±5.641	11.603±1.745	10.203±1.715	0.157	0.858
DEHP (μg/L)	18.216±3.206	17.470±1.679	22.141±2.028	3.292	0.108
BPA (μg/L)	2.789±0.790	2.800±0.735	3.281±1.006	0.326	0.734

Drinking Water Pollutant Levels

Compared with the control area, the concentrations of Hg, Pb, As, BPA, DEP, and DBP in

the drinking water of the polluted area were significantly higher ($P<0.05$); however, there were no significant differences in DMP and DEHP between the two areas ($P>0.05$) (Table 2).

Table 2. Concentrations of 8 Pollutants in Drinking Water ($\bar{X} \pm s$)

Indicators	Polluted Area (n=30)	Control Area (n=30)	t	P
Hg ($\mu\text{g/L}$)	0.752 \pm 0.244	0.452 \pm 0.121	2.466	0.039
Pb ($\mu\text{g/L}$)	17.200 \pm 8.311	7.200 \pm 1.202	2.663	0.029
As ($\mu\text{g/L}$)	31.025 \pm 6.580	21.212 \pm 6.599	2.355	0.046
BPA ($\mu\text{g/L}$)	0.160 \pm 0.113	0.003 \pm 0.002	3.116	0.036
DMP ($\mu\text{g/L}$)	38.189 \pm 2.272	38.760 \pm 5.804	-0.205	0.843
DEP ($\mu\text{g/L}$)	44.044 \pm 25.644	13.269 \pm 9.940	2.502	0.037
DBP ($\mu\text{g/L}$)	0.931 \pm 0.236	0.542 \pm 0.286	2.342	0.047
DEHP ($\mu\text{g/L}$)	12.487 \pm 1.555	11.750 \pm 0.224	1.049	0.351

Anthropometric Indicators and Sex Hormones of Boys

The anthropometric indicators and sex hormones of boys are shown in Table 3. Values of the anthropometric indicators including height, weight, bust circumference and skinfold thickness among the boys of the polluted area were found to be higher than those in the control area, but there was no statistically significant difference ($P>0.05$). However, the E2 and T were significantly lower in the control area than in the polluted area ($P<0.05$). (Table 3).

Table 3. Values of Anthropometric Indicators and Sex Hormones of Boys in Two Areas ($\bar{X} \pm s$)

Indicators	Polluted Area (n=30)	Control Area (n=48)	t	P
Height (cm)	139.00 \pm 11.	137.48 \pm 7.3	0.651	0.518
Weight (kg)	34.00 \pm 6.19	31.44 \pm 6.25	1.705	0.093
Skinfold(cm)	1.77 \pm 0.82	1.42 \pm 0.57	2.011	0.050
Bust Bircumr- ence(cm)	66.12 \pm 5.38	66.11 \pm 5.47	0.008	0.994
E2 (pg/mL)	29.27 \pm 5.73	25.45 \pm 3.94	3.208	0.002
T (ng/mL)	0.33 \pm 0.19	0.23 \pm 0.14	2.491	0.016

Anthropometric Indicators and Sex Hormones of Girls

A total of 76 girls participated in this study. Although there were no significant differences between the polluted and control areas in height, weight and bust circumference ($P>0.05$), the skinfold

thickness was significantly higher among the girls in the polluted area than in the control area ($P<0.05$). Compared with the control area, both the E2 and T were significantly higher in the polluted area ($P<0.05$). (Table 4).

Table 4. Values of Anthropometric Indicators and Sex Hormones of Girls in Two Areas ($\bar{X} \pm s$)

Indicators	Polluted Area (n=39)	Control Area (n=37)	t	P
Height(cm)	139.19 \pm 8.97	139.04 \pm 8.33	0.081	0.936
Weight(kg)	33.17 \pm 7.44	31.44 \pm 5.98	1.124	0.265
Skinfold thickness(cm)	1.99 \pm 0.77	1.56 \pm 0.51	3.036	0.003
Bust Circum- ference(cm)	66.12 \pm 7.69	65.68 \pm 5.52	0.289	0.774
E2(pg/mL)	31.52 \pm 5.54	27.14 \pm 4.36	3.798	0.001
T(ng/mL)	0.39 \pm 0.19	0.28 \pm 0.15	2.848	0.006

DISCUSSION

Drinking Water Influenced by SY River Pollution

Over the years, environmental protection departments at all levels have been growingly concerned about the Huai River pollution. Since beginning of management of the Huai River pollution in 1994, a decade's efforts have decreased the total emissions and improved the water quality to some extent^[10]. However, recent years have also seen gradual increase of the Huai River pollution. According to the monitoring data, about 50% of the Huai River Basin exceeded Class V standards in 2004 in terms of the surface water quality, which could directly affect local people living around the Basin^[1].

It could be known from this study that: 1) the contents of Pb and Hg exceeded Class V standards of surface water quality in each section and other indicators were more than III. 2) In addition to As, there were no significant differences in concentrations of concerned pollutants among the various sections. The above results suggest that the pollution of the SY River is still very serious and its self-purification capacity has disappeared.

In the current study, various pollutants, including heavy metals, polycyclic aromatic hydrocarbons and PAEs, were detected in the groundwater near the SY River at a depth of 20-50 m,

and even over 200 m^[11], justifying the argument that according to the water level and changes of riverbed structure, the river water can enter into the ground and form groundwater by different ways^[12-14]. The two main ways are as follows: one is the leaching-type which is the main way to supply groundwater in dry seasons, while the other is lateral seepage in rainy seasons. Because of the filtering action of soil, the concentrations of pollutants in groundwater were significantly lower than those in the river water, and a significant negative correlation was found between concentrations of pollutants in groundwater and the distance from the river^[14]. In this study, concentrations of Pb, Hg, As, DEP, DBP, and BPA in the drinking water of the control area were significantly lower when compared to the polluted area, suggesting that the drinking water of polluted area might have been contaminated by the polluted river via horizontal spread, vertical infiltration, as well as rainwater dissolution.

Hormone Levels of Children Affected by Pollutants in Drinking Water

Previous studies have shown that drinking water pollution has a direct relationship with the damage of human health^[5-8]. Children aged 8-13 years are in the second peak of growth and development. During this period, various systems are highly sensitive to environmental pollutants, especially the sexual development. And the hormone level is one of the important indicators of children sexual development. It could be learnt from this study that the serum concentrations of E2 and T were significantly higher in the polluted area than in the control area for both boys and girls. The difference in children's E2 and T levels between the two areas may be related to chemical pollution, which can interfere with composition and secretion of sex hormones. Other studies have revealed that Pb, Hg, As, PAEs, and BPA all have endocrine disrupting effects^[15-20]. They can influence and regulate the sex hormone levels by hypothalamus-pituitary-gonad axis and thus affect growth and development of children^[15]. Pb, Hg, As are highly toxic substances that can exist for a long time, and they are difficult to degrade in the environment and can affect the gonads of humans^[18]. PAEs can affect the ovarian secretion function in mice, showing the proposed role of estrogen^[17]. As an environmental endocrine disruptor, BPA also has dose-dependent estrogenic effects^[19].

Influence of Polluted Water on Anthropometric

This research also showed that the children in the two areas had no significant difference in the birth and feeding patterns, birth weight, maternal education, exposure to passive smoking exercise time, sleep time and so on according to analyses of the valid questionnaires. Such finding suggested that there were no genetics, nutrition, exercise and other confounding factors to impact on the children's growth and development in the two areas, and therefore, the two areas had comparable physical development of children.

Skinfold thickness is an important indicator used to infer the body fat content and determine the development of subcutaneous fat. Since different people have distinct genetic quality, living environment, diet and so on, the body fat distribution and its percentage in the total body weight are likely to show their own characteristics. A study showed that children in the puberty had higher subcutaneous fat content because their body had secreted a lot of estrogen^[21]. The result of this study indicated that the skinfold thickness of girls in the polluted area was significantly higher than that in the control area while the skinfold thickness of boys in the polluted area was higher than that in the control area but with no statistical significance. The reason may be that the children in the polluted area had a higher serum concentration of E2 as a result of the effects of EEDs. A high concentration of estrogen can make cells become larger and thus accumulate a lot of fat, resulting in a significant increase in skinfold thickness. As the puberty of boys was 1-2 years later than girls, the impact on girls may be larger than on boys. In view of this, the sample size shall be enlarged in future research. However, no significant difference was found among other indicators of physical growth. Findings of the present study are inconsistent with the results of another study^[22], according to which physical development indexes of children living in the polluted area were significantly lower than those in the control area, with the types and concentrations of pollutants in the water not clearly indicated. One reason for such inconsistency may be that the types and concentrations of pollutants in the water under study may be different from those in other literature, which only caused a change in hormone levels, but did not lead to morphological abnormalities.

In summary, the results of the present study show that the pollution of the SY River is still very

serious, and has affected the groundwater quality. Meanwhile, the pollutants in the drinking water have interfered with composition and secretion of children's serum sex hormones to some extent. In consideration of the limitation of this study, it is essential to replicate these findings in different villages near the SY River with larger sample sizes as well as more detailed information on children development and growth.

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