OBSERVATIONS ON SOME MINERALS AND B VITAMINS IN SEWAGE AND SLUDGES

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INTRODUCTION

SEWAGE contains proteins, fats, carbohydrates, minerals, vitamins and other substances, which make it a suitable medium for the growth and multiplication of micro-organisms, including pathogenic forms. The object of purification of sewage is to eliminate these substances and all pathogens from the medium and to make it as nearly as possible to the original composition of the water used. The work done in different industrial areas of the world over a period of many years to achieve this object has indeed led to practical treatment methods of reasonable efficiency. But it has been increasingly realised that a fuller knowledge of the constituents of sewage and their changes during treatment would be useful not only for a better assessment of the quality of the treated material but also for its better utilization. Attempts are, therefore, being made in different parts of the world to increase the efficiency of the treatment of sewage so that the treated material could be utilized particularly for augmenting the supply of water for agricultural and industrial purposes.[1,2]

SCOPE OF THE PRESENT INVESTIGATION

There is relatively more information on the nitrogen changes in sewage than on most other constituents of it. In recent years, however, the phosphates in sewage and effluents have attracted special attention because of their influence on excessive growth of algæ in the receiving waters.[3,4] Phosphates in waters have also a close bearing on the growth of entero-viruses[5] and bacteria such as *Salmonella typhosa*[6,7] and *Escherichia coli*.[6-8] But our knowledge regarding the nature and extent of removal of phosphorus from sewage during treatment by different methods is not satisfactory. The available information on other constituents of sewage and sludges, as on the following, is much less: sulphur,[9-12] iron,[9,13-17] calcium,[9,13,14,16,17] magnesium[9,13,14,16,17] copper,[13-15,17,18] zinc,[13-15,17,18] nickel,[15-17] chromium,[14-17] molybdenum,[18] cobalt[14,16,18,19] and vitamins.[12,19-28]

The work described here relates mainly to phosphates, sulphates, iron, calcium, magnesium, copper, zinc, nickel, chromium, molybdenum, cobalt and some B vitamins in sewage and the changes in their amounts during treatment by different methods, including the newly observed system of natural purification of flowing sewage at Bangalore. In view of the presence of cobalt and vitamin B_{12} in sewage and in view of the fact that cobalt forms an integral part of vitamin B_{12}, further studies on this vitamin in sewage and in sludges formed under different conditions were also carried out.

METHODS EMPLOYED FOR ESTIMATION OF THE MINERALS AND VITAMINS

The available methods for the determination of phosphorus, sulphur, iron, calcium, magnesium, copper, zinc, nickel and chromium were compared, and more suitable methods for sewage, sludges and effluents were selected. Determination of cobalt and molybdenum was carried out by colorimetric methods described by Sandell.[29] Phosphorus was determined by the method proposed by Fiske and Subbarow[30] and modified by King.[31] The sulphates in sludges were determined by gravimetric method and the sulphates in sewage and effluents were determined by the volumetric method, using benzidine.[32] Sulphide, iron, calcium, magnesium, copper, zinc, nickel and chromium were determined by standard methods.[29,32,33]

The Carr-Price method[34] was used for determining vitamin A, and the method using 2, 6-dichlorophenol indophenol was adopted for determining ascorbic acid.[34] These two vitamins were not detected in sewage, sludge, or effluents. Carotenoids were present in sewage and sludges (determined by colorimetric method[34]). Thiamine was determined by the thiochrome method.[34] Riboflavin was determined by the fluorometric method.[34] Niacin was determined colorimetrically by the cyanogen bromide method.[34] Vitamin B_{12} was determined microbiologically,[35] using *Lactobacillus leichmannii* A.T.C.C. 4797, *Escherichia coli* 113-3 and *Euglena gracilis* as the test organisms. Sewage and sludges were also examined microbiologically for folic acid, biotin and pantothenic acid, using *L. casei*, *L. arabinosus* and *L. plantarum*, respectively, as the test organisms.[34,36]

MINERALS AND B VITAMINS IN SEWAGE AND SLUDGES

Periodical analysis was carried out on (*a*) samples of domestic sewage from the sewage works at this Institute and from the natural sewage channels at Bangalore, (*b*) samples of sludges and effluents from the activated sludge plant and the septic tanks at this Institute, and (*c*) samples of sludges and effluents from treatments carried out under laboratory conditions. One set of results obtained under laboratory conditions are given in Tables I and II.

TABLE I

Results of analysis of sewage and effluents
(Average, expressed as mg /l.)

	Number of samples examined	Raw sewage*	Effluent from	
			Anaerobic treatment†	Aerobic treatment†
Phosphorus (P):				
Water-soluble	1658	7·5	7·5	1·2
Total ..	1658	11·1	10·7	1·7
Sulphate (SO_4)	178	57	41	55
Sulphide (S) ..	178	2	14	Nil
Iron (Fe) ..	105	10·1	7·2	2·9
Calcium (Ca) ..	102	45·3	36·3	37·5
Magnesium (Mg)	102	52·3	43·8	41·9

* The maximum amounts of copper, zinc, nickel and cobalt in the raw sewage were 0·05, 0·05, 0·01 and 0·8 mg./l. respectively. Only trace amounts of chromium and molybdenum were found in the sewage.

† These results of analysis of the effluents from treatments under laboratory conditions were similar to those from the septic tanks and the activated sludge plant, respectively, at the Institute. The effluents from both the treatments contained traces of copper, zinc, nickel, chromium and molybdenum.

Sewage and Sludges.—Raw sewage contains appreciable amounts notably of phosphates, sulphates, calcium, magnesium and iron. During treatment by the activated sludge process the sludge removed a large part of the phosphorus (about 90%) and iron (about 70%) from the sewage, leaving only small amounts of them in the effluent. In this process the sulphates were not removed to any considerable extent from the sewage. The percentage removal of calcium and magnesium in this process did not exceed 21.

Sewage also contains small amounts or traces of cobalt, copper, zinc, nickel, chromium, and molybdenum, and these were removed nearly completely by activated sludge and by septic tank sludge, and the effluents from these treatments generally showed only traces of these elements.

There are vital differences between these two sludges. While activated sludge contained only a trace amount of sulphide, the septic tank sludge contained a considerable amount of sulphide. The septic tank sludge, as compared to activated sludge, contained much less sulphates and phosphates.

The observations given in Table II also indicate that activated sludge contains relatively more thiamine, riboflavin, niacin and vitamin B_{12} than in sewage or in the sludge formed under anaerobic conditions. The amounts of these vitamins in activated sludge are comparable to the vitamin contents of liver, chicken, pork, bacon, sardines and salmons.[37] Activated sludge has been considered as one of the richest sources of vitamin B_{12}.[26]

TABLE II

Results of analysis of sewage and sludges
(Average of six analyses)

	Raw sewage solids	Sludge from	
		Anaerobic treatment	Aerobic treatment
Minerals (mg./100 g. of dry solids):			
Phosphorus (P) ..	500	680	1870
Sulphate (SO_4) ..	950	710	1800
Sulphide (S) ..	Trace	180	Trace
Iron (Fe) ..	500	680	880
Calcium (Ca) ..	1510	1700	1600
Magnesium (Mg) ..	1600	2150	1900
Copper (Cu) ..	4·0	50	50
Zinc (Zn) ..	4·3	52	58
Chromium (Cr) ..	Trace	3·4	3·4
Nickel (Ni) ..	,,	2·6	2·6
Molybdenum (Mo) ..	,,	4·8	5·5
Cobalt (Co) ..	30	95	98
Vitamins (mcg./100 g. of dry solids):			
Thiamine ..	580	650	800
Riboflavin ..	490	670	980
Niacin ..	2700	3200	4100
Vitamin B_{12} ..	16·4	9·6	75·4

Folic acid, pantothenic acid and biotin were present in the sewages and sludges. Qnantitative assay of these vitamins was not carried out.

The above results of analysis of the sludges from treatments under laboratory conditions were similar to those from the septic tanks and the activated sludge plant, respectively, except with regard to riboflavin which was comparatively less in the corresponding latter sludges.

Mention may be made of the earlier observation that pathogenic bacteria causing typhoid, cholera and dysentery are eliminated from sewage during purification by activated sludge process.[38] Some observations have recently been made on the use of activated sludge as a fertilizer for fish culture,[39] and more recent experiments at Bangalore have shown that the sludge could be used as a feed supplement for poultry.

Effluents.—The effluent from activated sludge process was practically free from sulphide. The effluent from anaerobic or septic tank treatment contained practically all the water-soluble phosphorus originally present in the sewage.

With regard to the other minerals studied, the effluents from the activated sludge process and anaerobic treatment were comparable except for iron which was considerably more in the effluent from the latter treatment.

Further Observations

Studies were also carried out on the extent of removal of the minerals from sewage during sedimentation, dilution with water in different proportions, chemical coagulation, filtration through granite chips of 1/2 inch size, sand and sand fractions of varying size[40] and during natural purification of flowing sewage at Bangalore.[41] During sedimentation and dilution of sewage, the mineral composition of the samples was not appreciably affected. The supernatant liquids from sewage samples coagulated with lime, alum and ferric chloride did not show any phosphate, but they showed the presence of the other minerals in varying amounts. Lime removed the maximum percentage (about 90 of the iron and nearly all the copper, zinc, nickel, chromium, cobalt and molybdenum).

Experiments with the filters indicated that the reduction or removal of the minerals is largely a chemical process in the early stages of the operation of the filters and that it is largely a biological process (except iron) in the later stages after the ripening of the filters. Similar changes were observed during purification of the sewage flowing down in natural channels at Bangalore.

Further experiments with activated sludge were also carried out by adding varying concentrations of chemicals such as mercuric chloride, zinc sulphate, copper sulphate, nickel sulphate, potassium chromate, sodium molybdate and cobalt sulphate. An observation of considerable interest and importance was the influence of an optimum concentration of cobalt on the enrichment of activated sludge vitamin B_{12}.[19,28]

Conclusion

The method of disposal or treatment of sewage to be chosen for a given place depends upon several factors, *e.g.*, the availability of land space, nearness to a watercourse and economic aspect. At the same time the proposed method should fulfil the requirements of environmental hygiene and sanitation.

The different methods of sewage treatment differ in their efficiency which is reflected in the final products. The liquid and solids from the activated sludge process show a high degree of purification. The sludge in this system removes from sewage most of the organic and mineral constituents to give rise to a clear effluent which could be safely used in agriculture or, after further treatment, in certain industries. The sludge thus produced is rich in proteins, minerals and B vitamins, notably vitamin B_{12}. It may be utilized as a fish feed or as a feed supplement for farm animals, such as chicks, in addition to its use as an excellent organic manure.

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MORPHOLOGICAL AND ANATOMICAL STUDIES IN HELOBIÆ

X. Trends of Specialization in Placentation in Helobiae *

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THE name Helobiæ was first used by Engler and Prantl[3] for a group of families including Potamogetonaceæ, Najadaceæ, Aponogetonaceæ, Scheuchzeriaceæ, Alismaceae, Butomaceæ and Hydrocharitaceæ. The seven families of the order are placed together chiefly on account of their aquatic habitat, presence of squamulæ intravaginales within the leaf bases, more or less complete absence of endosperm in seed, and enlarged embryo. However, the floral structures which are considered to be most conservative do not show any marked similarity. The vascular pattern of the flower and placentation also show a great variation.[7-14] The present study deals with some probable trends of specialization in placentation in the Helobiæ.

A wide range in placentation has been observed in different families of the order. They show median and apical (Potamogetonaceæ), basal (Najadaceæ, Scheuchzeriaceae, Alismaceæ); marginal and axile (Aponogetonaceæ); laminar or superficial (Butomaceæ); and parietal (Hydrocharitaceæ) placentation.

The marginal placentation is considered to be the simplest by the supporters of the classical concept (*see* Puri, 1952).[5] This is the condition in monomerous gynacea, which are generally believed to be most primitive and is shown by the primitive ranalian families where carpel is an involutely folded structure. Amongst Helobiæ this condition is met with in the Aponogetonaceæ where the carpels are free in the upper region and open in young condition like those of the primitive ranalian families. Each carpel bears two marginal rows of ovules and has a vascular supply of three bundles, one dorsal and two carpellary ventrals (Fig. 1). It has been visualized that the marginal placentation is the basic condition for the Helobiæ and the evolution seems to have progressed from this condition along three different lines leading to axile, superficial and basal placentation. Further specialisation in axile placentation has resulted in parietal placentation.

In one line of specialization the marginal placentation has given rise to axile placentation. This is seen in *Aponogeton* where the carpels though free are basally and adaxially connate. In the basal region the ovary is distinctly chambered, the two half placentæ are borne on the fused margins of the same carpel and derive their vascular supply from the ventrals of the same carpel (Fig. 2). Further the ventral bundles lie on the same radii as the carpellary dorsals and are inversely oriented. Thus the fusion of the carpels bearing ovules at their margins have resulted in axile placentation.

Further specialization towards parietal placentation which is prevalent in Hydrocharitaceæ has been brought about from axile placentation by more and more receding of placentæ towards the periphery so that the ventral bundles and their placentæ have occupied the peripheral position. The ventral bundles of the two adjacent carpels have also fused among themselves to form the vascular supply of parietal placentæ (Fig. 3). It may be pointed out here that in a number of families parietal