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Phosphorus-Containing Wastes of Southern Kazakhstan: Mineralogical and Microbiological Characteristics

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Abstract Wastes of the phosphate industry, located in the city of Shymkent, pose a serious threat to public health. The mineralogical composition of waste represented by such minerals as: pseudowollastonite (a-$CaO \times SiO_2$), followed by cuspidine ($3CaO \times CaF_2 \times 2SiO_2$), pyroxene ($CaO \times MgO \times 2SiO_2$), a-form of tricalcium phosphate ($Ca_3(PO_4)_2$), fluorite (CaF_2), vitacit (NaF). In a small number found silicoborate ($5CaO \times P_2O_5 \times SiO_2$), akermanite-$Ca_2MgSi_2O_7$, fluorapatite –$Ca_5(PO_4)_3F$, pyrite and calcite. The chemical composition of waste samples differs in the content of iron, calcium, fluorine, phosphorus, silicon, lanthanum, cerium, neodymium, and uranium. Found that the greatest number of microorganisms detected at a depth of 10-20 cm, which is explained by favorable conditions for life: air-gas conditions, humidity, lack of sun exposure. The population of microorganisms presented a heterotrophic, nitrogen-fixing, acidophilus iron oxidizing, nitrifying microorganisms and micromycetes. The results show the feasibility of bioleaching methods to the extraction of valuable components from the wastes.

Keywords Phosphorus-containing wastes, Mineralogical composition, Microflora

1. Introduction

The emergence of industrially polluted areas in the South of Kazakhstan is connected with the second half of the last century. For the anthropogenic impact on the environment in South Kazakhstan oblast (SKO) has been in first place in the Republic. Phosphorites of Karatau (the basic formula $Ca_5F(PO_4)_3$ were processed in two ways: heat and acid on the elemental yellow phosphorus, its compounds and fertilizers. As a result of the activities of the phosphorus plant in the territory of Shymkent has accumulated 0.5 million tons of hazardous waste. Accumulated during decades of waste phosphorus of the plant due to processes of urbanization were in the city. The processes of water and wind erosion contribute to environmental pollution by various compounds of phosphorus.

The use of biotechnological methods for extraction of valuable components from mineral waste is an environmentally safe method [3]. The most often use of acidophilic thionic bacteria *Acidithiobacillus ferrooxidans*. In research J. Zimmermann and Dott W. [13] it is shown using the biotechnological process of bioleaching and bioaccumulation of released phosphorus by newly developed population of bioleaching bacteria *Acidithiobacillus sp.* strains and polyphosphate (poly-P) accumulating bacteria, the AEDS-population (*Acidithiobacillus sp*. enriched digested sludge). Donati E. R. [2] studied the effect of immobilization thionic bacteria on clay carriers to increase their oxidative capacity.

In studies Sklodowska A, Matlakowska R [9] shows the possibility of bioleaching of valuable components in the neutral and weakly acidic media. The role of endomycorrhizal fungi to extract phosphorus from waste is shown in studies Adeleke et al [1].

Known studies related to the application of the micromycetes *Aspergillus niger* for obtaining boric acid from colemanite [7] prescribing such conditions of incubation temperature 25±20°C, the solids content of 5% with a fraction of 0.075 mm. The number of micromycetes 3×10^7 cells/ml, incubation time of 21 days. During the incubation micromycetes produces up to 90, 18% boric acid. *Aspergillus niger* used for bioleaching of cobalt and copper from cobalt concentrate [5], where the extraction of metals from cobalt concentrate is carried out in two strains of *Aspergillus niger* at a temperature of +240S at pH 3.5 for 15 days. The contents of the concentrate in the aqueous phase are 10 g/L. Extraction of cobalt is 82%, copper -98%. Yaraghi A., et al. [12] conducted studies on the bioleaching of zinc waste; Ten and Ting [10] investigated the possibility of bioleaching of electronic waste with the application of *Aspergillus niger*. This micromycetes was used for bioleaching of solid waste [11, 6] and the processing of spent catalyst from petroleum refining [8].

Analysis of the data shows a wide variability in the use of microorganisms for bioleaching of valuable components

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from mineral and industrial wastes. However, the conditions of the location of phosphorus-containing waste on the territory of Shymkent city dictate certain conditions: safety for the environment and human, economic profitability. To explore the possibility of application of methods of bioleaching of phosphorus-containing waste, the first task was to conduct environmental assessment of waste study of their mineralogical and microbiological characteristics.
In this context, the aims of the research, the results of which are presented in this article were physic-chemical and microbiological examination of phosphorus containing waste.

2. Material and Methods

2.1. Phosphorus-Containing Wastes

As the object of study, we used phosphorus-containing wastes, located in the amount of 1.5 million tons in the city of Shymkent in South Kazakhstan region. Wastes formed as a result of processing phosphate ore yellow phosphorus, mineral fertilizers, phosphoric acid, and sodium tripolyphosphate.

Visually phosphorus-containing wastes can be divided into four groups (Figure 1):

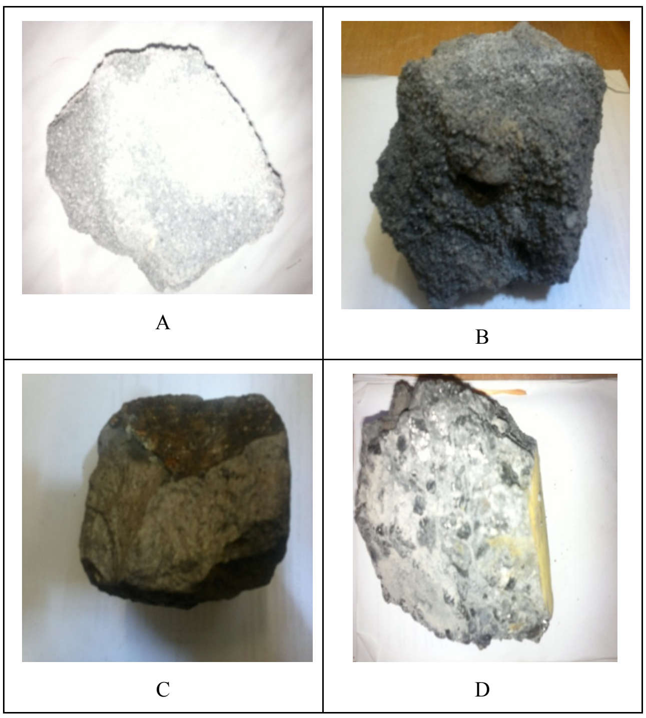

Figure 1. Different morphological types of phosphorus-containing waste. Where: A - grained, white, friable waste, B –dark loose granular waste, C waste heavy dark dense, D - dense gray waste

2.2. Microorganisms

An attempt was made to find out which of the microorganisms are found in phosphorus-containing wastes. For isolation of microorganisms samples of waste was ground in a mortar, were averaged. Selected 1 g waste, which was added to 100 g of sterile distilled water to make slurry. Next, 0, 2 ml of suspension was deposited on the solid surface of agar medium and distributed on the surface of the agar. The allocation of heterotrophs was carried out on the RPA, the following composition, g/l: Pancreatic hydrolysate sprat – 10, 05; NaCl -4, 95, agar-20, 0. The micromycetes were allocated on the Chapek medium following composition, g on 1 l of water: -30,0 sucrose or glucose - 20,0; $NaN0_3$-2,0; K_2HPO_4 - 1,0; $MgS0_4x7H_20$ - 0,5; KC1 - 0,5; $FeS0_4x7H_20$ - 0,1. nitrogen-fixing microorganisms on the Ashby medium, g/l: mannite (15% solution) – 27 ml; K_2HPO_4 – 0,2; $MgSO_4 \times 7H_2O$ – 0,2; NaCl – 0,2; K_2SO_4 – 0,1; $CaCO_3$ – 5; agar– 20.

The plates were rotated for easy mix up of the sample and the media. All plates were allowed to solidify on the bench. Each plated sample was duplicated. All the agar plates were transferred into an incubator at 25-27°C for 3 - 5 days. All the incubated plates were examined daily for colony growth. After incubation of all the plates, counts of the number of colonies in each plates was done with a hand tally counter. A mean of the count was obtained and multiplied with the appropriate diluting factor. The estimation of viable number of microorganisms TVC (total viable counts) in each sample was made in colony forming units (CFU):

$$TVC=1 \text{Weight of sample} \times N \times D, \quad (1)$$

Where:
N - average number of colonies,
D - dilution factor

For the isolation of nitrifying and thiobacteria used the method limit tenfold dilutions on liquid nutrient medium, e.g. for isolation acidophilus iron oxidizing bacteria used the Silverman-Lundgren medium (9K), g/l H_2O: $(NH_4)_2SO_4$ – 3,0; KCl – 0,1; K_2HPO_4 – 0,5; $MgSO_4x7H_2O$ – 0,5; $Ca(NO_3)_2$ – 0,01; $FeSO_4x7H_2O$ – 44,2; 10n H_2SO_4 – 1ml; for nitrifying bacteria 1 phase –Vinogradskii medium, g/l H_2O: $K_2 HPO_4$ - 1,0; $(NH_4)_2 SO_4$ - 1,0; $Mg SO_4 \cdot 7H_2O$ - 0,5; NaCI - 0,5.

The liquid nutrient medium was transferred into another incubator at 28-32°C for 3-5 days.

The accumulation of culture was placed in conditions of aeration in a rocking chair with amplitude of 180 rpm.

Identification and taxonomic affiliation of microorganisms were determined in accordance with Bergey′s Manual [2] on morphological, physiological and cytological characteristics of the colonies.

2.3. IRS Analysis

Physic-chemical study was performed using infrared spectroscopy on the spectrophotometer SPECORD 75 IR spectrometer and inductively coupled plasma with mass spectrometric detection Varian-820 MS(Australia), elemental composition by atomic adsorption analysis on the Analyst 800 spectrometer (Perkin-Elmer) and high performance liquid chromatograph Varian-Pro (Netherlands).

2.4. X-ray Diffractometry

Analysis was performed on an automated diffractometer DRON-4 with Cu Ka – radiation, β-filter. Conditions for recording of diffraction patterns: U=35kV; I=20 mA; scale: 2000 imp; time constant 2s; shooting Teta-2J; detector 2 deg/min. x-ray diffraction for semi quantitative basis difractograms performed on powder samples using the method of equal portions and artificial mixtures. Determined quantitative ratios of the crystal phases. Interpretation of diffraction patterns was performed using the data of filing of ASTM Powder diffraction file and the diffraction patterns of pure from impurities of the minerals. For the main phase were carried out the calculation of content.

2.5. Electron Raster Microscopy

A video recording of the material was carried out using the graphics card electron-raster microscope JSM 649LV manufactured by JEOL (Japan) with energy dispersive microanalysis system INCA Energy 350 OXFORD Instruments company (United Kingdom) related to system structural analysis of polycrystalline objects HKL Basis.

2.6. Statistical Processing

Statistical processing of the obtained results was performed by calculation of the arithmetic mean value and standard deviation. All definitions were carried out in 3-and 5-fold repetition. Data were processed using a personal computer IBM "Pentium" on the basis of packages of applied programs "Excel".

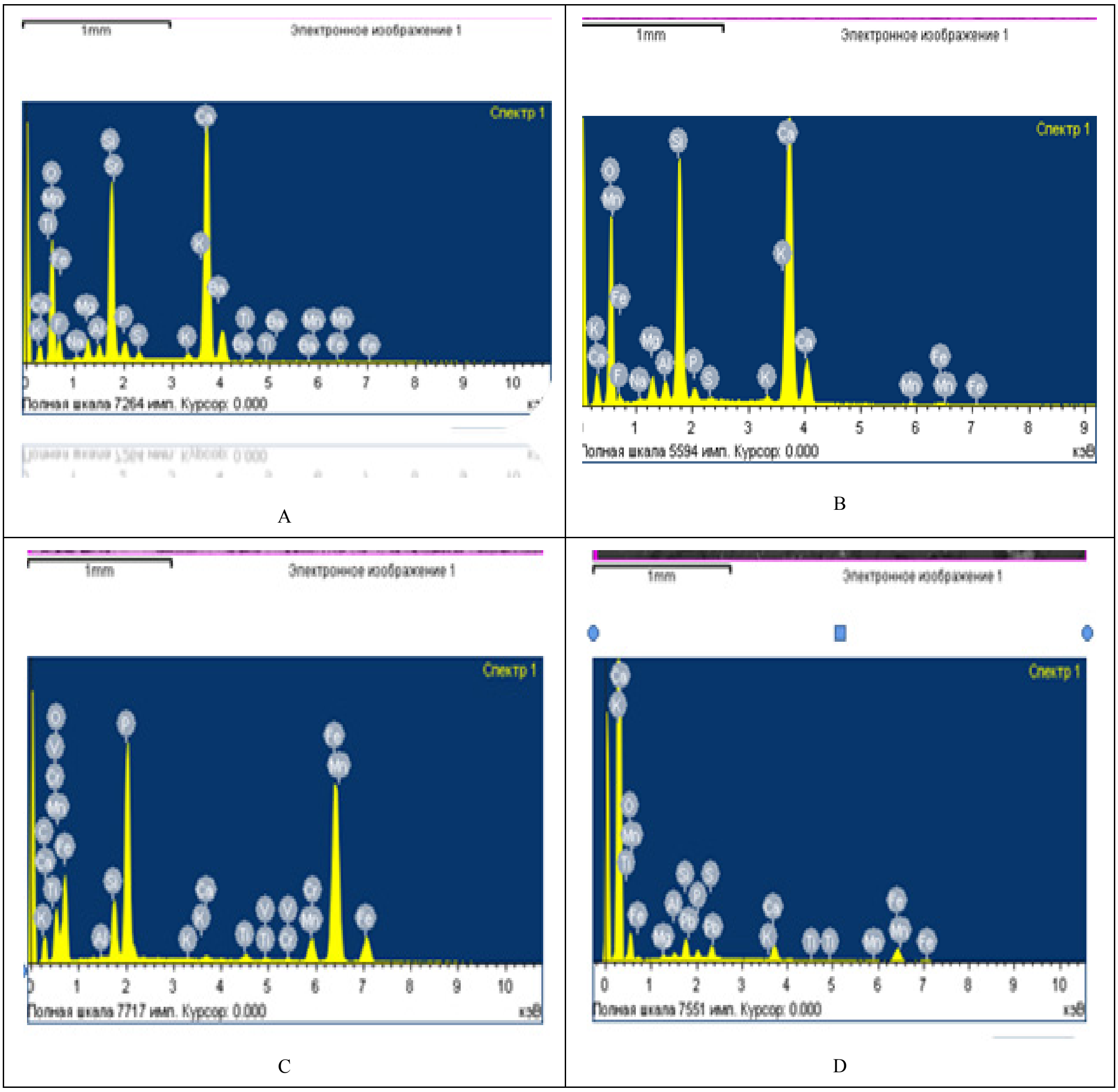

Figure 2. Data IRS tests, where: A - grained, white, friable waste, B –dark loose granular waste, C waste heavy dark dense, D - dense gray waste

3. Results and Discussion

It was found that the composition of phosphorus-containing wastes is presented, %: P_2O_5 -0, 63-3, 40, CaO - 44, 60-49, 35, SiO_2 - 40, 10-Of 43.54, Al_2O_3 -2, 46-of 3.48.

From the minerals most often determined by pseudowollastonite (a-CaO×SiO_2), followed by cuspidine (3CaO×CaF_2×2SiO_2), pyroxene (CaO×MgO×2SiO_2), a-form of tricalcium phosphate ($Ca_3(PO_4)_2$), fluorite (CaF_2), vitacit (NaF). In a small number found silicoborate (5CaO×P_2O_5×SiO_2), akermanite-$Ca_2MgSi_2O_7$, fluorapatite –$Ca_5(PO_4)$3F, pyrite and calcite. Electron microscopic examination and IRS-analysis shows morphological differences in the structure and composition of the waste (Figure 2).

The elemental composition of the analyzed samples are heterogeneous, as in samples A and B marked with fluorine in the amount of 4.59 and 2.01 weight % of that in samples C and D were not detected. At the same time, titanium, vanadium and chromium is present only in the sample C. the percentage of all samples is dominated by iron, calcium, phosphorus, silicon, in minor amounts detected magnesium, aluminum, sulfur and manganese. From rare earth elements in all samples detected lanthanum, cerium and neodymium, most of which are detected in the sample B. From radioactive elements marked the presence of thorium and uranium, the content of which ranges from 0,231 -20,088 µg / g and 8,419-259,389 µg / g respectively (Table 1).

Table 1. Impurities REE in the composition of phosphorus-containing wastes, µg / g

element	A	B	C	D
La 139	282,846	462,718	8,030	14,830
Ce 140	283,490	480,680	17,126	18,595
Nd 146	222,603	379,021	12,940	12,870

Microbiological examination of the samples indicated that the microorganisms are mainly concentrated in the upper horizons.

The greatest number of microorganisms detected at a depth of 10-20 cm, which is explained by favorable conditions for life: air-gas conditions, humidity, lack of sun exposure. So, the maximum number of heterotrophic microorganisms (3, 1±0, 25) x10^4 CFU observed at a depth of 0-10 cm, which increases to (1, 96±0, 09) x10^5 CFU at a depth of 10-20 sm. Deterioration of living conditions for microorganisms leads to a decrease in their numbers to (1, 61±0, 08) x10^2 and (0, 82±0, 06) x10 CFU at depths of 20-30 and 30-40 cm, respectively. This pattern persists for micromycetes and actinomycetes, the maximum number which are discovered in the horizon of 10-20 cm in the amount of (1, 02±0, 64) x10^3 and (0, 21±0, 01) x10^2 CFU respectively. In contrast, Acidophilus iron oxidizing bacteria in the greatest numbers occurring at depths of 20-30 cm in the amount of 10^3 cell/g, and the maximum number of Nitrifying bacteria 1 phase -10^4 cell/g observed in the upper layer of waste. The nitrogen-fixing bacteria observed at all the analyzed depths.

It is revealed that heterotrophic microorganisms belong to the genera Bacillus, Pseudomonas. Micromycetes presented genera *Aspergillus, Penicillium*. Nitrogen-fixing microorganisms present *Azotobacter* spp. The waste in the small number of identified acidophilic microorganisms, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*, which indicate a poorly running of microbiological processes. Nitrifying microorganisms present a *Nitrosomonas europeae*.

4. Conclusions

Found that phosphorus-containing wastes of southern Kazakhstan contain minerals such as: pseudowollastonite, followed by cuspidine, pyroxene, a-form of tricalcium phosphate, fluorite, vytlacit. In a small number found silicoborate, akermanite, fluorapatite, pyrite, and calcite. The chemical composition of waste samples differs in the content of iron, calcium, fluorine, phosphorus and silicon. In the samples marked by the presence of lanthanum, cerium, neodymium, and uranium.

Microbiological examination of the samples indicated that the highest number of microorganisms detected at a depth of 10-20 cm, which is explained by favorable conditions for life: air-gas conditions, humidity, lack of sun exposure. The population of microorganisms presented a heterotrophic, nitrogen-fixing, acidophilus, nitrifying microorganisms and micromycetes.

The studies found that the use of biotechnological methods in bioleaching of valuable components from phosphorus-containing waste is acceptable.

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