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Original Research

The Influence of Bacterial Reduction on Concentrations of Sulphates in Deep Aquifers in River Drainage Basin

Tomasz Kotowski[1]*, Aleksandra Burkowska[2]**

[1]Departament of Geology and Hydrogeology, Institute of Geography,
[2]Department of Environmental Microbiology and Biotechnology, Institute of Ecology and Environmental Protection,
Nicholaus Copernicus University, Gagarina 9, 87-100 Toruń, Poland

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Abstract

Our study attempts to determine the influence of bacterial reduction on sulphate concentrations in groundwaters. A buried valley in a southeastern section of the Gwda River drainage basin was selected for fieldwork near the towns of Wysoka and Bądecz. Deep aquifers in the area are known for their high sulphate concentrations that result from pyrite oxidation by the *Thiobacillus denitrificans* bacteria. Isotope compositions of SO_4^{2-} ions show ^{34}S enrichment at lower sulphate concentrations, which might indicate bacterial reduction processes. Theoretical curves of SO_4^{2-} isotope composition and ion concentration change, drawn using a Rayleigh formula and the results of the determination of $\delta^{18}O$ (SO_4^{2-}) and $\delta^{34}S$ (SO_4^{2-}) suggest widely varying intensities of the sulphate reduction processes. It can be estimated that bacterial reduction eliminated between 30% and in excess of 50% of the initial concentrations of SO_4^{2-} ions in individual cases.

Keywords: groundwaters, sulfate, bacteriological reduction, isotopic composition

Introduction

The study area is located near the villages of Wysoka and Bądecz in a southeastern section of the drainage basin of the Gwda River along the western section of the Polish Baltic coast. Highly dynamic changes in concentrations of the SO_4^{2-} ion were detected in a deep aquifer in Pleistocene deposits lying within a buried valley. Moreover, the deep Pleistocene aquifer within a buried valley were found to contain higher concentrations of chloride ions than the changeability range of these ions observed before a buried valley area [1]. The ascent of dilution brines from Mesozoic aquifers is the major process responsible for the increase of the Cl^- ions. However, the isotopic composition of SO_4^{2-} ions and the chemical composition of dilution brines indicated other source of sulphates than the ascending brines [2]. The higher concentrations of SO_4^{2-} ions had been caused by the oxidation of syngenetic pyrite [3]. Taking into account the fact that in the study area the groundwater is used for human consumption, these processes in aquifers occasionally cause serious problems. For instance, they can induce secondary dissolution of rock-bearing minerals and they directly influence the concentrations of Mn^{2+}, Ca^{2+}, and Mg^{2+} ions and the amount of iron rust ($Fe(OH)_3$). Such processes in aquifers can have a detrimental effect on quality and treatment of water. The study area is located in the western section of the Polish Baltic coast, where similar buried valleys interconnected with different aquifers occur over a wide area. Hence, understanding formation processes of chemical composition in a deep aquifer in Pleistocene deposits within the study buried valley is important, not

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*e-mail: kotowski.tomasz@gmail.com
**e-mail: wodkow@umk.pl

Table 1. The well's parameters, isotopic composition of sulfates, concentration of SO_4^{2-}, Cl^-, and O_2, and temperature.

No.	Date	Location	Depth of aquifer roof and well screen[1)]	SO_4^{2-}	Cl^-	O_2	$\delta^{34}S$ NBS-127	$\delta^{18}O$ V-SMOW	temp.
			[m]	[mg/l]	[mg/l]	[mg/l]	[‰]	[‰]	[ºC]
3	07.08.07	Debrzno	149/177	7.9	6.4	0.59	4.57	10.48	10
8	03.11.06	Bądecz	103/108	105.0	51.8	0.20	-2.66 -3.29	7.52	10.5
9	07.08.07	Wysoka	40/67	63.2	37.3	0.30	0.33	6.12	9.8
10	03.11.06	Młotkowo	70/84	11.2	10.8	0.35	6.95	13.04	11

[1)] Deep of midpoint of screen.

only for understanding chemistry of groundwater, but also for the preservation of water resources.

Microbiological processes of sulphate reduction are widely discussed in published research [including 4-6]. Detailed discussions are found in numerous groundwater studies. For instance, $\delta^{34}S$ values in groundwater are used to identify sulfate provenance [7-9]. Moreover, it has been shown that the process of sulphate reduction can be quantitatively estimated (under some assumptions) by applying a Rayleigh distillation model using sulfur isotopic ratios $\delta^{34}S$ and concentrations of dissolved SO_4^{2-} [8, 10-12]. Some previous studies reported occurrences of opposing redox reactions, such as sulfate reduction and sulfide oxidation within one confined (locally unconfined) aquifer system [13], or found that redox cycling of sulphur occurred where there was a low net rate of sulfate reduction [14]. Of these, numerous groundwater studies focused on detailed descriptions of bacterial sulphate reduction in polluted aquifers (e.g. contaminated by different kinds of hydrocarbons [15-17], Yamanake et al. [12] undertook studies in a system with very similar hydrogeological conditions to those studied here (i.e. confined sandy aquifers built with Pleistocene and Neogene deposits, tectonic faults within a valley zone, and scale of study area) and also found that both sulphide oxidation and sulphate reduction took place in the aquifer system.

Building on our relatively good knowledge of the local hydrogeological conditions and following isotopic and microbiological investigations, an attempt was made to determine the influence of microorganisms on sulphate concentrations in the local groundwaters. The research presented in this paper is a continuation of previous studies of the development of the chemical and isotopic composition of the local groundwaters [1-3].

Hydrogeological Conditions

The Pleistocene deposits include between two and three aquifers of different origin. The deep Pleistocene aquifer in the oldest sandy sediments of Pleistocene age is found only in the buried valley. The deep the Pleistocene aquifer is interconnected with a Miocene aquifer to form a common groundwater flow system. The bottom of the Pleistocene aquifer rests on top of low-permeability Neogenic deposits. Local Miocene deposits had been replaced, partly or entirely, with Pleistocene deposits. The water-bearing layers sampled (Table 1) are directly interconnected hydraulically. The deep Pleistocene aquifer was found on very similar elevations to the Miocene aquifer elevations. Despite distinct lithology and genesis of the aquifer deposits, the deep Pleistocene aquifer was considered together with the Miocene aquifer as a common element of groundwater flow system. Within a buried valley area there is a tectonic fault found directly over a salt anticline in the underlying Mesozoic strata. The scheme of geological and hydrogeological conditions is presented in Fig. 1.

A more extensive discussion of hydrogeological conditions in the study area is available in earlier studies of the area [1, 2].

Methodology and Results

Three samples were taken from the buried valley in order to determine the isotopic composition of the SO_4^{2-} ions in the deep Pleistocene aquifer. A single sample was taken from the Miocene level for comparison purposes. The sampling sites are shown in Fig. 2A.

The concentration of Cl^- and SO_4^{2-} ions found in samples from Bądecz and Wysoka (Fig. 2B) is much higher than in all other samples from the buried valley. The area's average concentration of Cl^- ions is 19 mg/l and that of SO_4^{2-} ions is 24 mg/l [1]. The increase in the SO_4^{2-} ion concentration seen in Fig. 1B is non-linear and does not appear to bear any relationship to groundwater exploitation volumes at Bądecz which, according to an oral account, remained similar throughout 2004-07.

Tritium concentrations were either only slightly above the error margin (±0.3) or zero. Furthermore, no typical evidence of anthropogenic impact was found, which rules out any significant addition of human-polluted waters [18].

Isotope compositions of sulphates (Fig. 3B) were determined at the Mass Spectrometry Laboratory, Institute of Physics, UMCS of Lublin. VSMOW was adopted as the isotopic reference for oxygen and hydrogen, while the NBS-127 reference was adopted for sulphur and oxygen in sulphates [19]. Sampling site characteristics are summarized in Table 1.

Fig. 3 shows the isotopic concentration of $\delta^{34}S$ (SO_4^{2-}) in the samples as a function of the SO_4^{2-} ion concentration (part A) and the variability of $\delta^{34}S$ (SO_4^{2-}) and $\delta^{18}O$ (SO_4^{2-}) (part B). There is a clearly visible drop in the SO_4^{2-} ion concentration and an increase in the $\delta^{34}S$ (SO_4^{2-}) value, which according to several authors would suggest an effect of bacterial reduction of SO_4^{2-} ions [6, 20, 21].

Microbiological processes of sulphate reduction are widely discussed in published research [4-6]. The phenomenon of isotope fractionation of the SO_4^{2-} ions occurs during a dissimilation reduction reaction. An SO_4^{2-} ion is bound with ATP (adenosine-5'-triphosphate) to form a coordination compound. This is followed by a reaction leading to the breaking of a bond between sulphur and oxygen and the

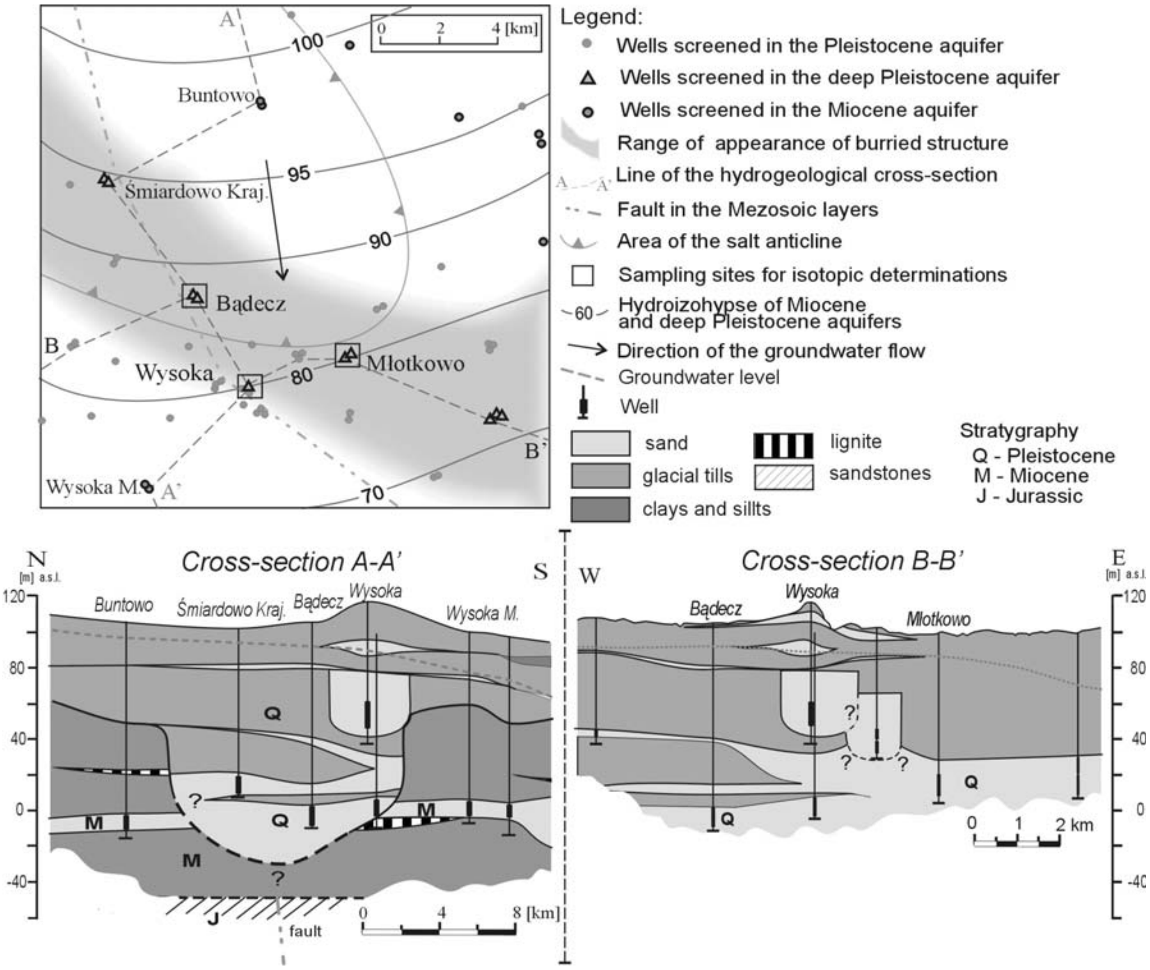

Fig. 1. Scheme of hydrogeological condition of study area.

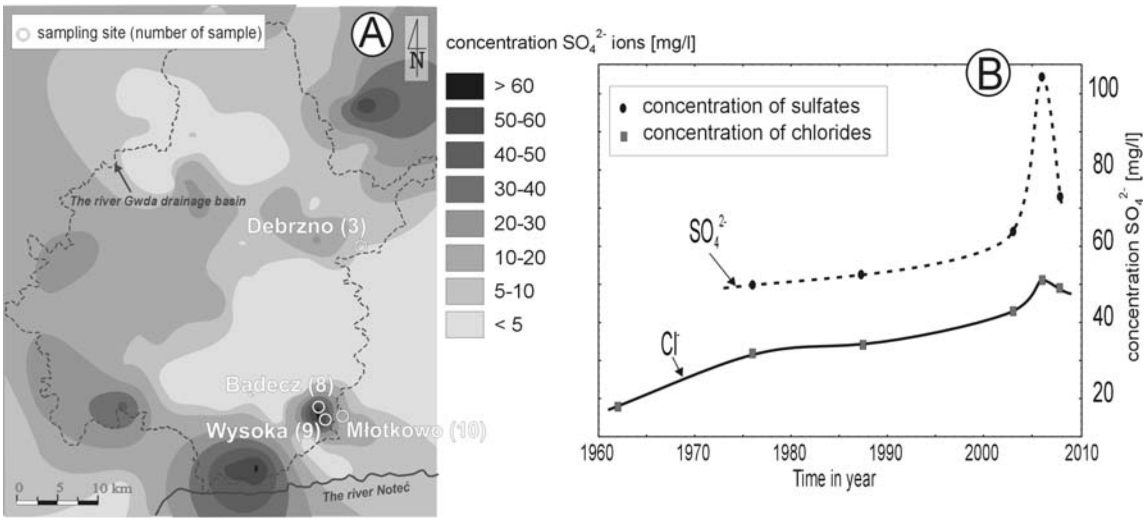

Fig. 2. Location sampling site and map concentrations of SO_4^{2-} ions in study area against Gwda catchments and the time variability of chloride and sulfate ion concentrations for samples from Badecz.

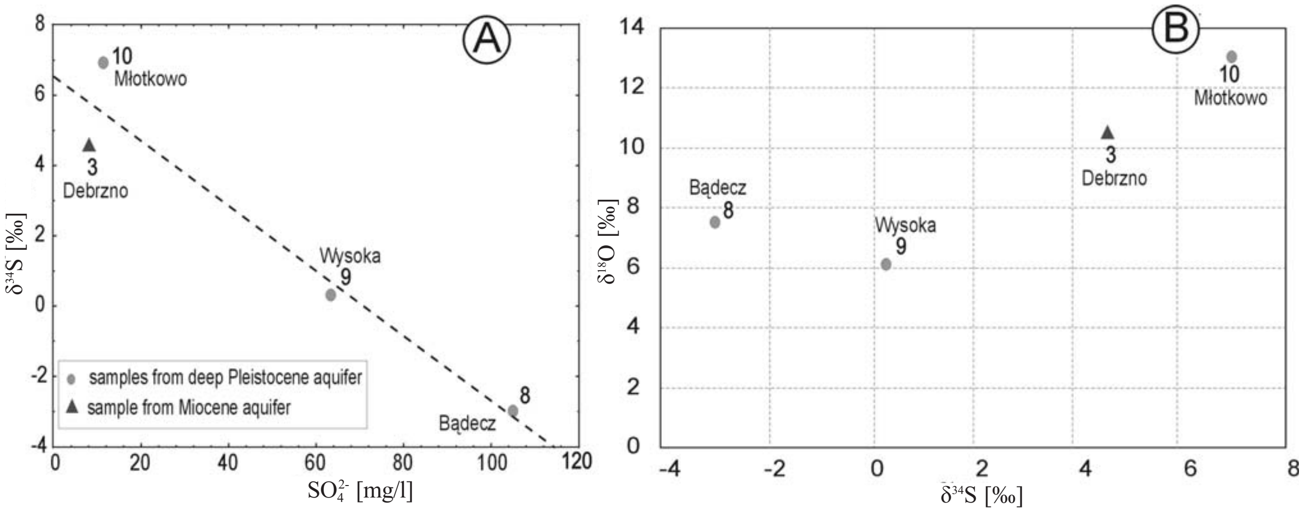

Fig. 3. The variability of the isotopic composition of sulfates vs. concentration of sulfates ions (part A) and the plot of value $\delta^{34}S$ (SO_4^{2-}) and $\delta^{18}O$ (SO_4^{2-}) (part B).

transformation of the SO_4^{2-} ion into an SO_3^{2-} ion. During this phase of the reaction, there is no isotopic effect. In the second phase (i.e. isotope exchange reactions), the SO_3^{2-} ion is reduced to H_2S and an isotopic fractionation (i.e. an equilibrium re-distribution of isotopes among species) occurs as in reaction (1), below:

$$^{32}SO_4^{2-} + H_2{}^{34}S \xleftrightarrow{k} {}^{34}SO_4^{2-} + H_2{}^{32}S \qquad (1)$$

...where k is the reaction rate constant.

Isotope exchange reactions can be viewed as a subset of kinetic isotope reactions – under fixed, adequate conditions [22]. At isotopic equilibrium, the forward and backward reaction rates of any particular isotope are identical. Although the isotopic exchange reactions can take place under more-or-less equilibrium conditions, more typically the system is out of chemical equilibrium (e.g. evolution of SO_4^{2-} ions during groundwater flow) or the products become partially isolated from the reactants (e.g. diffusion of dissolved H_2S, precipitation of sulphides). Under these conditions, the isotopic compositions of reactant are affected by an additional kinetic isotope fractionation and the isotope reactions may, in fact, be partially (or purely) unidirectional. Kinetic isotope fractionation is caused by the fact that the covalent bond between the isotopically light sulphur and the also light oxygen is easier to break. As a result, there is a large difference in the reaction speed between the light and heavy sulphur isotopes. Kinetic fractionations, especially unidirectional ones, are usually larger than the equilibrium fractionation factor for the same reaction in most low-temperature environments. Numerous isotope reactions occurring in nature (e.g. sulphate reduction, methane oxidation, nitrification) can be modelled with Rayleigh-type models. The Rayleigh model can also be applied to equilibrium closed systems and kinetic fractionations [22].

In this study the Rayleigh distillation model was applied under the following assumption:

1) The study-confined aquifer is an open system that continuously interacts with its environment.
2) Products are continuously removed from the system (probably as a result of diffusion – see the direction of groundwater flow in Fig. 1).
3) The "resource" of SO_4^{2-} ions is limited (note the high dynamic of changes of SO_4^{2-} ion concentrations and the short period of these changes, about 3-4 years; Fig. 2A.

Microbiological sulphate reduction processes are known to cause the greatest isotopic variability of sulphur in nature. Other processes involving the formation of sulphur compounds in the natural environment, such as oxidation, cause a much less significant isotopic effect than bacterial reduction [23].

The microbiological analysis of ground water samples was performed at the laboratories of the Department of Water Microbiology and Biotechnology, Nicolas Copernicus University of Toruń. The methodology of determination of the bacterial count and the results obtained are extensively presented by Kotowski and Burkowska [3].

Interpretation of Results

Negative values of $\delta^{34}S$ (SO_4^{2-}) determined in the study suggest that the oxidation of sulphur compounds at lower oxidation levels may be the source of the sulphates found at Bądecz. Considering that there were no anthropogenic influences, it was concluded that the higher SO_4^{2-} ion concentrations had been caused by the oxidation of syngenetic pyrite [3]. The mineral is ubiquitous in Oligocene and Neogene sediments in the study area [24, 25]. The pyrite oxidation process may occur in oxidizing conditions according to the reaction below [26, 27].

$$FeS_2 + 3\tfrac{1}{2}H_2O + 3\tfrac{3}{4}O_2 \rightarrow Fe(OH)_3 + 2SO_4^{2-} + 4H^+ \quad (2)$$

In low- or no-oxygen environments pyrite oxidation can proceed by way of microbiological denitrification processes in the presence of nitrates, as shown in the reaction below [28, 29].

$$5FeS_2 + 14NO_3^- + 4H^+ \rightarrow 5Fe^{2+} + 10SO_4^{2-} + 2H_2O + 7N_2\uparrow \quad (3)$$

In light of the very low oxygen concentrations in deep aquifers (Table 1) and the presence of nitrate that has been detected [4], the bulk of the pyrite oxidation is most likely to follow the pattern shown in reaction (3). The high value of $\delta^{18}O$ (+0.752%) determined in the sulphates from Bądecz exceeds the values of $\delta^{18}O$ observed in sulphates that originated from pyrite oxidation, which range from –0.8% to +0.5%. The effect is probably caused by the isotope fractionation of oxygen in oxidation reactions [30].

Samples taken at Bądecz were microbiologically tested for the *Thiobacillus denitrificans* bacteria and high counts were found (210 NPL per 100 cm^3) [3]. *Thiobacillus denitrificans* is a strict autotroph and in anaerobic conditions can oxidize pyrite through a denitrification reaction in which the oxygen is obtained from nitrates by reducing them to nitrogen as in reaction (3).

As no sulphate-reducing bacteria were detected in the samples from Bądecz, it is difficult to determine the influence of microbiological reduction processes on the isotope composition of sulphates in this case. Taking into account the very high dynamics in the SO_4^{2-} ion concentrations (Fig. 2B), this influence is minor, despite the high fractionation factor (1.5-2.5%) observed during these reactions [6]. This could be explained by the fact that the fractionation factor is inversely proportional to temperature, which is low in normal ground waters and in the sampling area is ca. 10ºC (Table 1). Bacterial reduction reactions proceed relatively slowly in these conditions despite the high fractionation factor. The sharp increase of the SO_4^{2-} ion concentrations observed at Bądecz suggests that the isotope compositions of the dominant sulphates are either unmodified or modified only slightly.

The value of $\delta^{34}S$ (SO_4^{2-}) in the sample from Wysoka is clearly higher than in the one from Bądecz (Fig. 3A), which probably indicates that the sulphates from Wysoka are enriched in heavy sulphur isotopes as a result of reduction processes. While no bacteria that reduce SO_4^{2-} ions were detected in the Wysoka sample, a single sample is not sufficient to rule out the presence of such bacteria in the past, as was also the case with the Bądecz sample. Indeed, during regular three-year surveys of the underground Omulewski aquifer Niewolak et al. [31] found bacteria reducing the SO_4^{2-} ions in 50-80% of the samples taken from various wells. In each well the bacteria occurred periodically and their occurrence and count were highly variable in time.

In view of the fact that non-bacterial sulphate reduction processes require high temperatures and are characterized by a low isotope fractionation factor [23], one must reject the possibility that sulphate reduction is taking place solely through abiotic factors in this instance.

The count of *Thiobacillus denitrificans* detected in the sample from Wysoka (150 NPL per 100 cm^3) was similar to that from Bądecz [3]. However, there are no pyrites in the Pleistocene formations and therefore no pyrite oxidation can occur in the Wysoka area. For this reason it can be determined with a high degree of certainty that the high concentration of SO_4^{2-} ions found at Wysoka comes from recharge by groundwaters found near Bądecz that contain such high concentrations. This hypothesis is confirmed by similar values of $\delta^{18}O$ (SO_4^{2-}) and $\delta^{34}S$ (SO_4^{2-}) found in the samples from Bądecz and Wysoka (Fig. 3B). Also, the Cl^- ion concentration found in the Wysoka sample was clearly higher than the hydrochemical background, thus suggesting a considerable volume of groundwater inflow from the Bądecz area [18]. Groundwaters from the Mesozoic levels at Bądecz were found to ascend through the tectonic fault and this is also where the highest concentration of Cl^- ions was found in a deep Pleistocene aquifer within a buried valley [1]. Taking all this into account, it is assumed that the isotopic composition of SO_4^{2-} ions in the sample from Wysoka was the result of their modification by the bacterial reduction of sulphates.

Values of $\delta^{34}S$ (SO_4^{2-}) found in samples taken at Debrzno and Młotków are markedly higher than typical values determined in sulphur compounds produced as a result of the transformation of previously reduced forms (e.g. pyrite, marcasite). These values, however, are not high enough to assume that the process of dissolving evaporites could be the main source of SO_4^{2-} ions. According to Clark and Fritz [32], one can also reject the hypothesis that the sulphates originated as dissolved maritime evaporites when sulphate $\delta^{34}S$ is less than +10‰. It was therefore concluded that the clear shift visible in $\delta^{34}S$ (SO_4^{2-}) values toward positive in samples from Debrzno and Młotków suggests an enrichment of the sulphates with heavy sulphur isotopes, most likely as a result of microbiological sulphate reduction processes.

Theoretical Model of Bacterial Reduction

Despite the low number of samples tested, an attempt was made to estimate the original (primary) $\delta^{34}S$ value in the water samples from Debrzno, Młotków, and Wysoka. A Rayleigh formula was used:

$$\delta = \delta_o + 1000(\alpha - 1)\ln\frac{C}{C_o} \quad (4)$$

...where:

δ – original value of $\delta^{34}S$ (SO_4^{2-})

δ_o – currently observed value of $\delta^{34}S$ for SO_4^{2-} ions in the samples tested

α – isotope fractionation factor during bacterial reduction

C – current concentration of SO_4^{2-} ions from tests

C_o – assumed original concentration of SO_4^{2-} ions

Using formula (4), above, and the fact that SO_4^{2-} ion concentrations went down as a result of bacterial reduction, theoretical $\delta^{34}S$ variability curves were drawn as a function of the reversed concentration of SO_4^{2-} ions (Fig. 4).

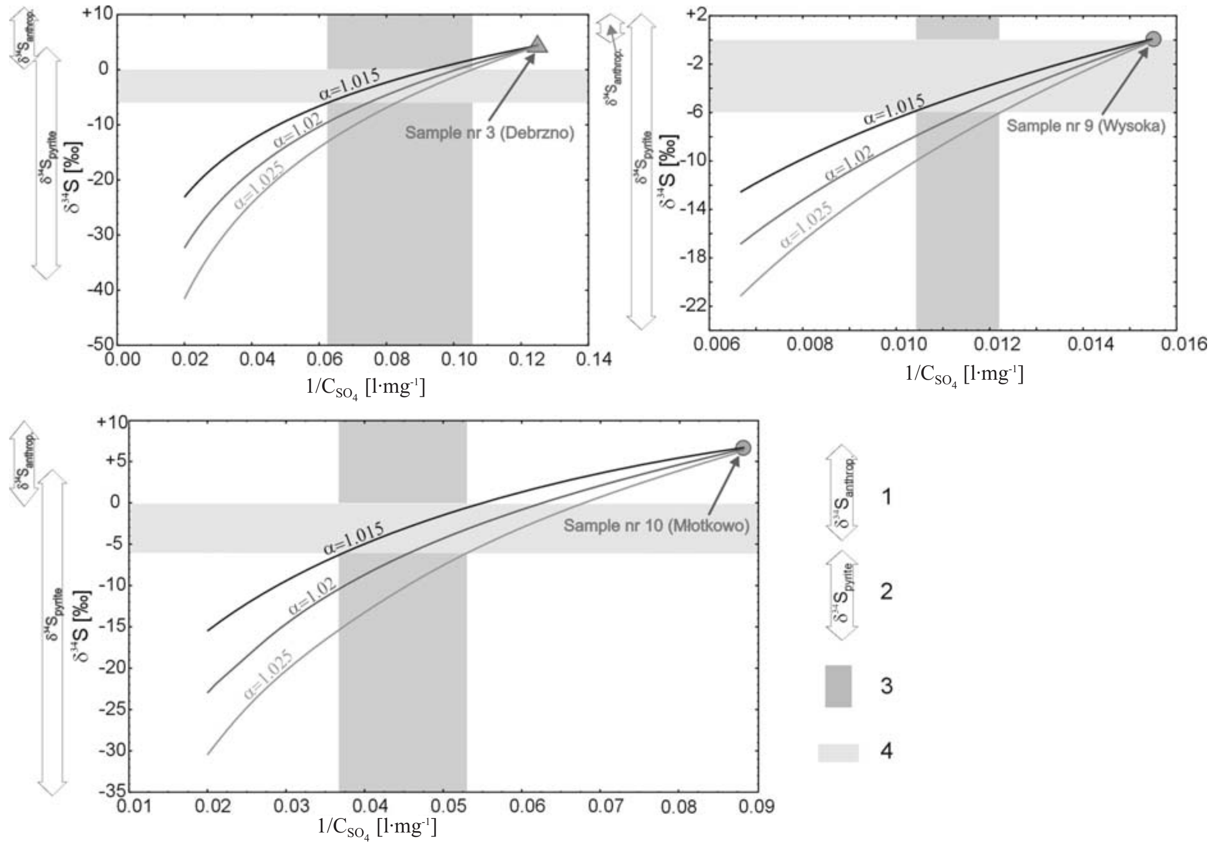

Fig. 4. The variability of $\delta^{34}S$ vs. function of inverse concentration of SO_4^{2-} ions.
1 – the average values of $\delta^{34}S$ (SO_4^{2-}) of anthropogenic parentage as a result of fossil fuel burning [after 33, 34]
2 – the average values of $\delta^{34}S$ (SO_4^{2-}) of natural geological parentage as a result of pyrite oxidation [after 30, 35]
3 – the assumed range of variability concentration of SO_4^{2-} ions
4 – the calculated range of variability of $\delta^{34}S$ (SO_4^{2-})

The hydrogeochemical background values (n=101) determined for deep aquifers in the Gwda drainage basin were used for an assumed range of concentration variation of SO_4^{2-} ions produced as a result of pyrite oxidation [18]. The theoretical curves shown in Fig. 4 were drawn for isotope fractionation factors ranging from 1.5‰ to 2.5‰. These are the most frequently observed values in groundwaters [6]. In particular, when H_2S is removed from groundwaters (for instance as a result of sulphide precipitation) the isotope fractionation factor often fails to reach its maximum value [23].

In drawing the theoretical curves (Fig. 4) the authors took into account the fact that SO_4^{2-} ions produced by pyrite oxidation in the deep aquifer near Bądecz have an isotope composition that is either unchanged or only slightly changed. For this reason it was assumed that the isotope composition of SO_4^{2-} ions found in the sample from Bądecz should be regarded as being characteristic of, or close to characteristic of, an isotope composition of SO_4^{2-} ions produced by pyrite oxidation in physico-chemical conditions typical of the study area.

The analysis of the theoretical curves indicates that the original SO_4^{2-} ions had a lighter isotope composition, which was typical of SO_4^{2-} ions produced by pyrite oxidation [30].

By taking the $\delta^{34}S$ values in the sample from Bądecz as a reference point one can estimate that the original concentration of SO_4^{2-} ions in Debrzno and Młotków did not exceed 20 and 30 [mg/l], respectively. In this way it can be concluded that bacterial reduction processes effectively removed half of the original concentration of these ions. The situation at Wysoka is somewhat different, as the recharging groundwater from Bądecz was most likely to be responsible for the mixing of two groundwater components. Lab testing for the concentration of Cl^- and SO_4^{2-} ions performed in October 2007 showed similar results of 49.6 and 73.2 [mg/l], respectively, at Bądecz and 38.3 and 72.8 [mg/l] at Wysoka. Additionally, isotopic composition testing for sulphates from the two sites at Wysoka and Bądecz yielded results that showed more similarity between them than in comparing them to all other sites. The theoretical original concentration of SO_4^{2-} ions in the sample from Wysoka did not exceed 100 [mg/l], a similar result to that at Bądecz. Therefore, the drop in the SO_4^{2-} ion concentration at Wysoka in comparison to Bądecz can be put at ca. 30 [mg/l].

The chemical composition of groundwater noticeably changes during groundwater flow through a buried valley area (Fig. 5).

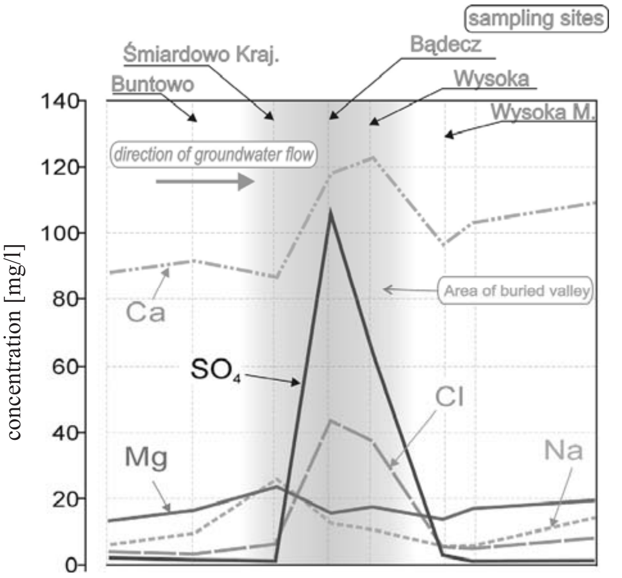

Fig. 5. Variability of selected ion concentrations in the deep Pleistocene and Miocene aquifers.

Pyrite oxidation is the process responsible for the increase of SO_4^{2-} and Ca^{2+} ions within a study area. The lowering of pH as a result of this process has not been observed in the deep Pleistocene aquifers due to buffering by calcite dissolution. This process directly influences the concentrations of Ca^{2+} ions. At once, the higher concentrations of iron ions (not shown on Fig. 5) were found within a buried valley [1]. Most probably it is linked with the oxidation pyrite. The sharp decrease of the concentrations of SO_4^{2-} related to sulphate reduction. It has indicated the highly dynamic and large intensity of these processes.

The increase of concentration of Cl^- ions is mainly connected with the ascent of dilution brines, via fault, from the deep Mesozoic aquifers. The tectonic fault within a study area (Fig. 1) is unrelated with the oxidation of pyrite. In the study area, the tectonic faults occur in Mesozoic rocks only and they discontinue the surfacing of terrain (not intersected Neogene and Pleistocene deposits). Moreover, soft Pleistocene and Neogene deposits (mainly clays and tills) have effectively muffled tectonic dislocations. Hence, the oxidation pyrite by flow of unconfined groundwater accompanied by dissolved oxygen into confined aquifers via fault is not possible. Indeed, the currently observed concentration of dissolved oxygen in deep Pleictocene aquifers is low (Table 1).

The alteration of the chemical composition of groundwater is connected with two major separate processes: the oxidation of pyrite and ascent of dilution brines. This has been described in detail by Kotowski et al. [1-3] and is not discussed here.

Conclusions

It is generally concluded that the SO_4^{2-} ions in the study had been and may continue to be produced in deep aquifers of the study area primarily as a result of bacteria-assisted pyrite oxidation. A more extensive discussion of the origin of sulphate in the area is available from a study by Kotowski and Burkowska [3].

No sulphate-reducing bacteria were detected in the samples, but the isotope composition of the samples and the generally low concentration of SO_4^{2-} ions (often <1 [mg/l]) in the study area indicate that intensive bacteria reduction processes used to, and may continue to, occur at a regional scale. Additionally other research suggests that sulphate-reducing bacteria often occur periodically and their occurrence and counts are highly variable in time [31].

Taking into account the theoretical curves the interpretation of the currently observed concentrations of SO_4^{2-} ions in the samples tested would suggest that the intensity of sulphate reduction (and pyrite oxidation) processes varied widely.

It can be estimated that bacterial reduction has eliminated between 30% and 50% or more of the original SO_4^{2-} ion concentration in the cases analyzed. While in light of the small sample size the assumptions adopted undermine slightly the train of thought adopted when using the Rayleigh formula, the results obtained may provide a departure point for further study.

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