

ASSESSMENT OF NATURAL AND ARTIFICIAL SOURCES OF SELENIUM IN THE ENVIRONMENT OF MOLDOVA REPUBLIC

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ABSTRACT

High levels of Selenium (Se) have been detected in the shallow groundwater systems of a rural region in Moldova. Selenium concentration in groundwater exceeds the Maximum Allowable Limit by 1.5 to 24 times (range: 15 to 240 µg/L). The population in this region is exposed to these high Se levels through direct consumption of groundwater as drinking water. We analyzed the Se content of environmental media (soil, rock, sediments and groundwater) in order to identify possible sources of Se in this region. Samples were taken from a variety of regions with different land-use including both rural agricultural regions and urban areas. The Se concentration in groundwater from rural regions is controlled by the composition of the aquifer material. In industrial regions Se may enter the groundwater through leakage from refrigerate ponds at the South Moldavian Power Station. Our data demonstrate the critical role played by organic matter in the accumulation and migration of Se.

INTRODUCTION

Selenium (Se) is an essential trace metal. In humans, Se deficiency has been linked to Keshan Disease, which has been observed in low-selenium areas of China (Fordyce et al., 2000a; Fordyce et al., 2000b; Johnson et al., 2000; Tan et al., 2002). Selenium deficiency may also affect thyroid function given that the element is essential for the synthesis of active thyroid hormone (Levander, 1997). Researchers also believe that selenium deficiency may worsen the effects of iodine deficiency on thyroid function. Consequently, adequate intake of selenium in the diet enhances protection against some of the neurological effects of iodine deficiency (Fordyce et al., 2000a). A large percentage of the rural population in Moldova relies on groundwater as a source of water for domestic and agricultural uses. High levels of Se in the shallow groundwater systems rural Moldova (Tan et al., 2002) pose a great danger to the majority of the inhabitants in these areas. In some regions, Se concentration in the groundwater exceeds the Maximum Allowable Limit (MAL) by over 24 times (up to 240 µg/L). The U.S. Food and Drug Administration (1982-86), has set a tolerable upper intake level for Se at 400 µg/day for adults to prevent the risk of developing selenosis (poisoning caused by ingesting Se). Given that the Recommended Dietary Allowance of Se is 70 µg/day, there is a immense concern over the exposure to such high concentrations of this trace element. While the records high levels of Se in the groundwater of rural Moldova exist, the impact of this metal on exposed humans, livestock and aquatic ecosystems in exposed regions is not known. Our study focuses on the potential sources of Se to groundwater in the affected regions and provides the necessary data for subsequent toxicity testing programs. Potential sources of Se in the environment include both natural and/or anthropogenic sources. For example, atmospheric deposition of Se and other trace elements has negatively impacted soil quality in Norway (Lag & Steinnes, 1974; Lag & Steinnes, 1978; Steinnes, 2001). Se concentration in soil depends also on parent rock Se concentration and regional climate. Potential anthropogenic sources of Se include local industrial activities and discharge of household waste. Understanding the source of Se to the environment, particularly Se in soils, is critical given Se in soils can be transferred to plants and ultimately to humans.

Se occurs in a variety of oxidation states (Se^{2+} , Se^0 , Se^{4+} and Se^{6+}) each of which may be present in soils. Mobility and bioavailability of Se are strongly dependent on its oxidation state and the binding capacity of the soils. Therefore, information about total Se concentration is not sufficient and so Se speciation is required. Our study, assessing the Se species concentrations in soils improves our understanding and evaluation of Se cycling, flux and balance in geocosystems in addition to providing critical data on Se impact on human health. We investigated the geochemistry of Se in different environmental media (soil, rock, sediment, and groundwater) from several regions of Moldova. We studied the total Se concentration in groundwater, soil, rocks, sediments and industrial wastes as well as speciated the Se in soil, rock and waste samples showing high total Se.

MATERIALS AND METHODS

Sample Collection

In total, 142 solid samples (soil, sediments, and rock) and 85 groundwater samples were collected from three study sites (Fig. 1; Carpineni village, Chisinau city, and Cuciurgan). Carpineni village is a rural area with agriculture as the main form of land-use while Chisinau is an urban area whose landscape has been greatly altered. The third study site, South Moldavian Power Station, is located in Cuciurgan. To characterize Se concentration in Carpineni area, Chisinau city and the Cuciurgan, soil samples from the bottom soil horizon (0.0-10.0 cm) were collected and analyzed. The Carpineni site samples were further categorized into three different land uses: arable lands, vineyards, and township to explore any differences in Se concentration amongst the three land uses. In total, we had five groups of soil samples from the three study sites. Bottom sediments were collected from small rivers and their tributaries flowing through the selected areas and as well as from refrigerate ponds at Power Station using a Van Veen grab sampler. Rock samples were collected from exposed sections and boreholes. Finally, groundwater samples were collected from drinking water wells and boreholes in all three regions. To identify the potential Se sources to groundwater as well as differences in groundwater Se between regions we divided the groundwater samples into three categories: wells on the boundary of Chisinau city; boreholes on boundary of Chisinau city; and, wells in Carpineni village.

Analytical Methods

Groundwater samples were preserved using concentrated HCl (3 mL/L). After sampling, soil samples were dried at room temperature, sieved using a 1.0 mm screen and prepared using the standard procedures (PerkinElmer, 2000; US Environmental Protection Agency, 1996). Two methods of digestion for total Selenium determination were used: heating with the solution of Aqua Regia, and heating in the presence of HNO_3 and H_2O_2 . A 5 mL aliquot of digested solution was pipetted into a 50 mL volumetric flask to which concentrated HCl (25 mL) and deionized water (15 mL) were added. The flask was placed in a water bath for 15 minutes at 900 C to reduce selenate to selenite. After cooling, the solution was brought to volume with deionized water. Se concentrations (Tables 1 – 3) in all samples were analyzed by hydride generation (FIAS-400) atomic absorption spectrophotometry (Perkin Elmer Aanalyst800). All samples were analyzed in triplicate.

Analytical Conditions

The accuracy of our data depends upon the methodology used. Due to the diversity of samples to be analyzed we assessed the effect of matrices on Se quantification by HGA-AA. The measurement conditions for HGA analysis were: characteristic concentration – 0.2 $\mu\text{g/L}$; method detection limit – 0.1 $\mu\text{g/L}$; absolute concentration – 1×10^{-4} $\mu\text{g/L}$. The percent recovery upon addition of 10 $\mu\text{g/L}$ Se to a standard of known concentration is shown in Table 4. These results show that the difference between Selenium concentration in standard solution and the samples with different matrixes (soil, sediments, water) did not exceed 5 % level, and are thus in limits of statistic errors.

Sequential extraction

Soil, sediments, and rock samples were sequentially extracted as summarized in Figure 1 (Bujdos et al., 2000). An aliquot (2.0 g) of the dried sample was placed in a 100 mL polyethylene (PE) bottle and phosphate buffer (25 mL) was added. The mixture was shaken for 1 hour at room temperature on a horizontal shaker. The mixture was centrifuged (20 minutes at 3000 g) and the supernatant decanted into a 100 mL PE bottle. After addition of 5 mL of deionized water to the residue, the mixture was thoroughly mixed manually (3

minutes), and centrifuged (20 minutes at 3000 g), and the supernatants were combined. Extraction of the mineralized organic Se fraction involved transfer of the phosphate extract (5 mL) into a 50 mL volumetric flask containing 10 mL of 0.1 M $K_2S_2O_8$. The solution was placed in a water bath (90⁰ C) for 30 minutes. After cooling, 25 mL of concentrated HCl was added and selenate reduced as described above. An aliquot of the 0.1 M $K_2S_2O_8$ solution was added to the residue and the bottle was placed in a water bath (90⁰ C) for 2 hours. The mixture was centrifuged (20 minutes at 3000 g). The supernatant was decanted into a 100 mL PE bottle, and 5 mL of deionized water added. The mixture was thoroughly mixed manually (3 minutes), and centrifuged (20 minutes at 3000 g). The supernatants were combined. The residue was digested by scheme (B) as shown in Figure 1 and described above. This method is a more reliable procedure for soils containing variable amounts of organic matter and having different chemical properties. All extractions were done in duplicate.

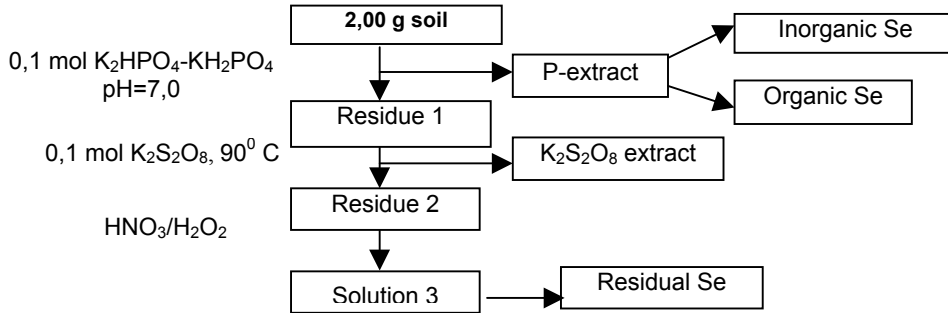


Fig. 1 Scheme of Sequential extraction procedure

RESULTS AND DISCUSSION

Selenium in Groundwater

Groundwater Se concentrations showed a normal statistical distribution. 17.5 % of the samples exceeded the maximum allowable limit (MAL) of 10 µg/L with highest proportion of low Se occurring in samples taken from wells in Chisinau City (Fig. 2). Similarly high Se concentrations occurred in borehole samples from Chisinau. We attribute these low Se concentrations to aquifer material (Quaternary alluvial deposits). The aquifer consists of loess loam, sandy loam, sand, and clay. Our results indicate that groundwater from the Carpineni region contain high levels of Se. Only 20 % of these samples have a Se concentration below the MAL (10 µg/l). The remaining 80% of the samples exceeded the MAL and several samples contained up to 330% of the MAL. Our findings are in agreement with previous studies in this region (TACIS, 2000)

Statistics	Chisinau wells	Chisinau boreholes	Carpineni wells
N (valid)	40	23	21
Mean	6.470	5.748	16.159
Std. Error of Mean	0.778	0.610	1.889
Median	4.850	5.700	18.200
Mode	2.90	3.30	1.10
Std. Deviation	4.920	2.945	8.655
Variance	24.207	8.671	74.901
Skewness	2.582	1.543	0.143
Std. Error of Skewness	0.374	0.481	0.501
Minimum	2.20	2.00	1.10
Maximum	28.00	14.00	33.40

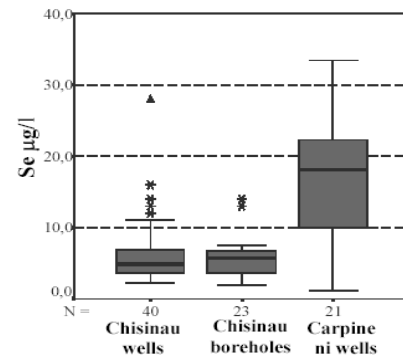


Fig. 2 Se content in groundwater of different regions from Moldova

Selenium in Soils

Soil Se concentrations exhibit no significant differences (Fig. 3) between the study sites with the highest mean Se concentration of 0.20 mg/kg occurring in arable soil samples from Carpineni and the lowest mean

value of 0.14 mg/kg in Chisinau city soil samples. Only a few soil samples exceed a value close to the deficiency threshold of 0.125 mg/kg. Our results indicate that the land use in the rural region of Carpineni village does not influence Se concentration in the topsoil.

The concentration levels defining Se deficiency and toxicity are well defined (Tan et al., 2002). The mean value of the total Se in the Moldovan soil samples falls within the marginal Se exposure interval (0.125 - 0.175 mg/kg) with the exception of soils collected from Carpineni arable lands, which have a mean value of 0.200 mg/kg. 27% of the Chisinau soil samples fall below the Se deficiency threshold of 0.125 mg/kg, 33% of the Carpineni vineyard soils, 39% of the Carpineni township soils, 27% of the Carpineni arable land soils, and 36% of the Cuciurgan power station soils also fall below the Se deficiency threshold. Other researchers have reported Se concentrations similar in range to those found in our study. Soils collected from forested land in the Slovak Republic have a mean total Se of 0.400 mg/kg while in Sri Lanka Se concentrations in agricultural soils ranges from 0.226 to 1.124 mg/kg (Fordyce et al., 2000a). Other studies include measurements of soils in China, which show a range in Se concentrations from 0.097 to 0.549 mg/kg (Fordyce et al., 2000b; Johnson et al., 2000; Steinnes, 2001), and Norwegian forest soils with a range in Se of 0.300 to 1.000 mg/kg (Lag and Steinnes, 1974; Lag and Steinnes, 1978). The total Se concentration in soils from regions throughout Moldova tends towards the deficiency threshold.

Statistics	Chisinau	Carpineni vineyard	Carpineni township	Carpineni arable land	Cuciurgan Power Station
N	15	15	28	26	14
Mean	0,143	0,171	0,164	0,200	0,151
Std. Error of Mean	0,0169	0,0213	0,0234	0,0211	0,0161
Median	0,138	0,145	0,130	0,170	0,145
Std. Deviation	0,0656	0,0824	0,1239	0,1077	0,0604
Variance	0,0043	0,0068	0,0153	0,0116	0,0036
Minimum	0,054	0,089	0,052	0,081	0,071
Maximum	0,305	0,377	0,711	0,483	0,272

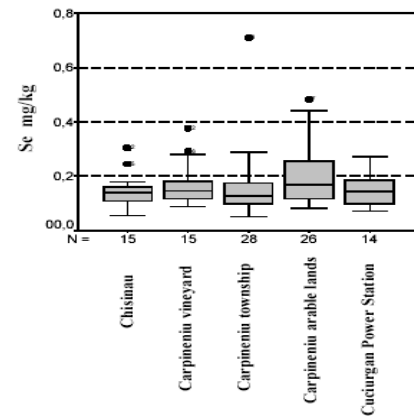


Fig. 3 Se content in top soil samples of different regions from Moldova

Selenium in Rocks

Se concentration in soil depends on the content of this element in the parent rock. For the Carpineni and Chisinau soils the parent material is Quaternary loess loam. Loess is a continental sedimentary rock and contains low concentrations of Se (Figure 4). The mean concentration is 0.056 mg/kg (range: 0.032 to 0.078 mg/kg). Thus loess loam cannot be a source of Se in soils and groundwater in this area. Similar conclusions were made in a study of loess rocks in China and other regions (Fordyce et al., 2000b; Johnson et al., 2000; Steinnes, 2001). Underlying the Quaternary loess is Sarmatian horizon, which is separated on three layers with the upper horizon characterized by subcontinental depositional conditions and is comprised of sands and clays. The Middle Sarmatian layer is characterized by marine depositional conditions (clay and limestone). Sediments of the Middle Sarmatian were deposited at a time of active volcanism on the continents and so clays within this unit (also dolomite, limestone, etc) contain thin layers with volcanic ash materials. This horizon could be a source of Se to soils and groundwater. The deep Early Sarmatian layers does not serve as an aquifer material in our study area.

The geological section in our study areas is characterized by the occurrence of Sarmatian layers below the Quaternary loess. The Se concentration of rocks from the upper Sarmatian layer is shown in Figure 4. The sandy clay of the upper Sarmatian horizon has a mean Se concentration of 0.098 mg/kg. Based on the range of Se concentrations in this unit we discount the possibility that this unit is the source of Se to soils and groundwater. Two samples of the clay from the middle Sarmatian layer from Carpineni township had high Se concentrations (1.622 and 1.733 mg/kg). These samples were collected an exposure within a ravine and so of this clay is weathered. It is possible that this layer is the source of Se in the groundwater given that clay seams occur close to the erosion level. We analyzed clay samples of middle Sarmatian layer from the borehole, which was drilled on the territory of Chisinau (local coordinates X-16460; Y-22700). The results of

this determination are presented in the table 1. We can see that dusty sand layers have higher Selenium concentration in the comparison of the all clay bed. This fact confirms our conclusion that middle Sarmatian rocks can be as one of possible Selenium source for Ground Water in investigated regions.

Statistics	Quarternary Loess Loam	Neogene sarmatian Sandy Clay	Lapusna River Sediments	Cuciurgan Power Station sediments
N	10	10	10	14
Mean	0,056	0,098	0,161	1,317
Std. Error of Mean	0,0045	0,0169	0,0204	0,1467
Median	0,055	0,084	0,149	1,284
Std. Deviation	0,0145	0,0534	0,0644	0,5487
Variance	0,0002	0,0029	0,0042	0,3011
Skewness	-0,327	0,245	0,714	1,008
Std. Error of Skewness	0,687	0,687	0,687	0,597
Minimum	0,032	0,023	0,073	0,514
Maximum	0,078	0,181	0,282	2,654

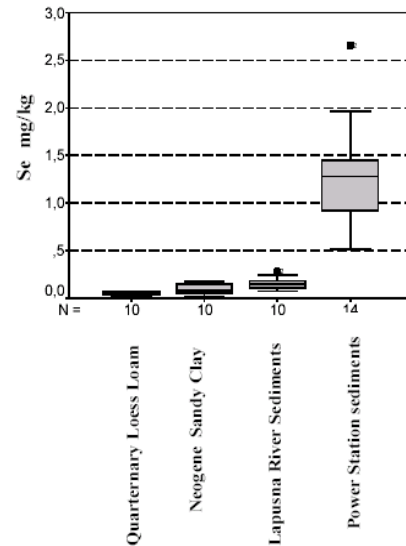


Fig. 4 Se content in rocks and sediments

Table 1: Selenium concentration in neogen clay of middle Sarmatian layers

Depth, m	Geological section		Statistics		
	Clay	Fine sand with ash materials	Statistics	Clay	Fine sand with ash materials
	Se mg/kg	Se mg/kg		Se mg/kg	Se mg/kg
8.0	0.334	0.541	Mean	0.442	0.896
12.0	0.399	0.542	Median	0.429	0.875
15.0	0.574	0.825	Std. Deviation	0.083	0.337
21.0	0.408	1.392	Minimum	0.334	0.541
24.0	0.488	1.150	Maximum	0.574	1.392
26.0	0.450	0.925			

Selenium in Sediments

Selenium content in sediments from the Lapushna River and its tributaries in Carpineni region and from a small stream in Chisinau City were similar to Se concentrations in the soil at these locations. We suggest that runoff does not play a significant role in Se transport from the soils and its accumulation in the sediments. However, a sample from a tributary of the Lapushna River in Carpineni had a very high Se concentration (3.333 mg/kg). This high Se concentration is probably due to weathering of the Neogene Sarmatian clay that is exposed in this tributary. In this region Se may enter the sediments through runoff during precipitation events. The Selenium content in sediments from the refrigerant ponds of Power Station is high with a mean concentration of 1.317 mg/kg. Currently, this power station uses Masut and natural gas for power generation. However, coal was used prior to 1985 and this likely explains the high Se concentrations in these pond sediments.

Selenium speciation in soils, sediments and rocks

We measured the concentration of Se species in solid samples to enhance our understanding of Se geochemistry in this region. For this investigation, we chose samples with extreme Se concentrations (Table 2). We chose the following samples: C25 – Carpineni arable topsoil, C15 – Carpineni forest topsoil, C35 – weathered Neogene middle Sarmatian clay, 5Cu – Cuciurgan dry refrigerate pond sediment, 7Cu – Cuciurgan wet refrigerate pond sediment, and A4 – Lapushna sediment. Determination of the total Se by scheme (A) and (B) gave similar results (Table 2 and Figure 5). Se concentrations were slightly higher using Scheme A than Scheme B. The sum of determined forms by sequential extraction also is close to schemes (A) and (B). The bulk of the Se in the soil samples (C25 and C15) was residual Se (Table 3). Inorganic Se

after P-buffer extraction was the last abundant Se species. Only the Carpineni forest soil had a detectable level on inorganic Se. The Neogene clay is dominated by organic Se extracted using $K_2S_2O_8$ while the inorganic less soluble form of Se was not detectable. Sediments from refrigerate ponds (5Cu and 7Cu) at the Cuciurgan Power Station were dominated by the organic form extracted using $K_2S_2O_8$. The next dominant form was organic Se extracted using P-buffer. The least abundant form in these sediments was inorganic Se. Organic Se extracted using $K_2S_2O_8$ dominates in stream sediments.

Table 2. General characteristics of samples for the form determination of Selenium

Sample #	Humus %	Granulometric content %			Total Se concentration mg/kg	
		Sand	Dust	Clay	Scheme A HNO_3+HCl	Scheme B $HNO_3+H_2O_2$
C25	2.02	73.32	14.28	12.40	0.483	0.388
C15	1.93	81.48	9.44	9.08	0.967	0.626
C35	0.21	39.04	23.84	37.12	1.682	1.733
5Cu	1.22	90.12	4.53	5.35	2.350	2.140
7Cu	1.43	96.76	0.80	2.44	2.654	2.415
A4	1.85	81.92	9.60	8.48	3.333	3.122

Table 3: Selenium concentration in different forms of solid phase

Index	P extract (inorganic)		P extract (organic)		$K_2S_2O_8$ extract (organic form)		Residual form extract of $HNO_3+H_2O_2$		Sum of Forms mg/kg	Recov. %
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%		
C25	n/d	n/d	0,049	12,9	0,113	29,8	0,218	57,3	0,380	87
C15	0,034	5,4	0,121	19,1	0,137	21,6	0,342	53,9	0,634	80
C35	n/d	n/d	0,060	3,5	1,053	60,4	0,628	36,1	1,741	102
5Cu	0,471	17,7	0,604	22,7	1,559	58,6	0,026	1,0	2,660	119
7Cu	0,300	11,6	0,598	23,1	1,390	53,7	0,300	11,6	2,588	102
A4	n/d	n/d	0,417	14,4	1,746	60,3	0,733	25,3	2,896	90

Light soluble Se concentrations are only detectable in samples with a high concentration of P-extractable Se. Samples with P-extractable Se below 15% do not have detectable inorganic Se. Organic Se species dominate samples with high total Se. Total soluble Se (P- and $K_2S_2O_8$ extractable) depends on the total Se concentration (Fig. 6). The residual Se concentration depends on mineralogy. The extraction of P buffer at pH 7.0 (Fig. 2, Scheme A) releases different forms of Se: soluble adsorbed, ligand-exchangeable and plant protein bound Se (Bujdos et al., 2000). It is apparent that this extraction scheme removes a significant portion of the humic and fulvic acids, which, at pH 7.0, are partially soluble. These complex organic compounds can directly react with Se species (selenite and selenate) and bind Se into their structures. Samples with

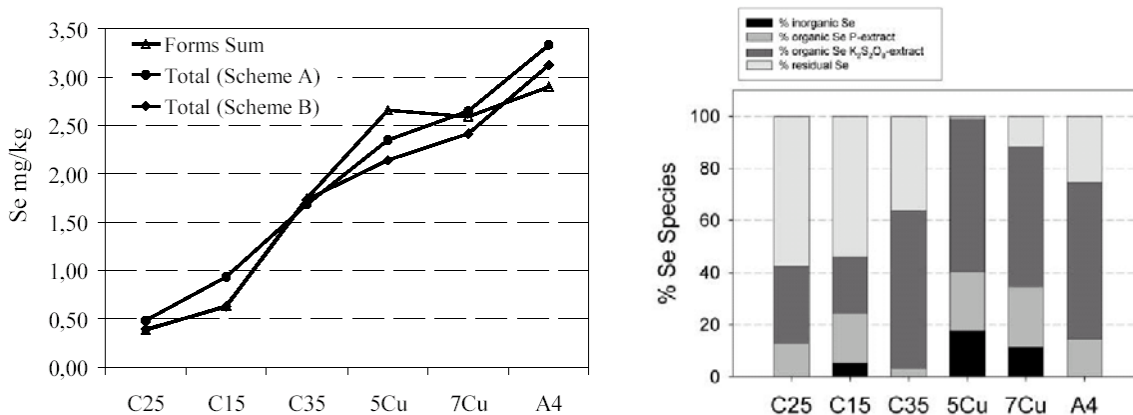


Fig. 5 Se content in different forms of solid phase of soil, rock and sediments

organically bound Se that was not extracted at pH 7.0 releases Se into solution by the oxidation in the 0.1 M $K_2S_2O_8$. The residual form characterizes the remaining Se, which is composed of plant-bound Se and amorphous Se_0 . Soil samples with comparatively high residual Se (C15, C25) contain Se in the un-

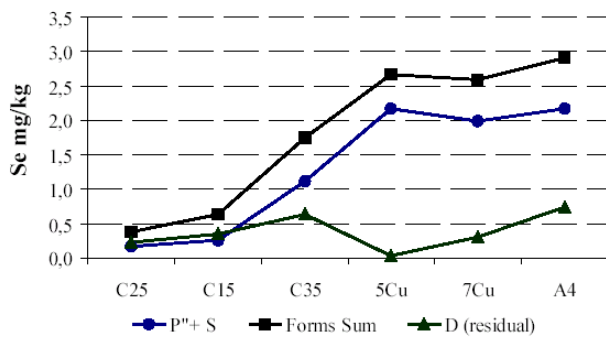


Fig. 6 Correlation between organic forms (P^{II} + S) sum of all forms and residual form in samples after sequential extraction procedure

decomposed plant tissue. Similar conclusions can be made about the sediments from rural Carpineni (A4) and the wet Cuciurgan refrigerate pond (7Cu) both of which contain a high percentage of plant-bound. High residual Se in the Neogene clay sample (C35) is associated with amorphous mineralized Se.

Conclusions

The high Se concentration in groundwater depends on the Se content of the aquifer material. We showed that the Neogene middle Sarmatian clay contained abundant Se and could be the source of Se to groundwater. Another possible source of Se to groundwater in the Carpineni region could be the refrigerate ponds of South Moldavian Power

Station. Our data suggests that Se from the sediments in refrigerant ponds could, though seepage, lead to increases in groundwater Se concentration. The accidental release of this element into the groundwater can take place through deposition of fly ash such as in Carpineni however the contribution of this source to the Se budget is minor. The sporadic distribution Se contaminated soils in Carpineni suggests that fly ash contribute to the soil Se only in specific regions. Soils in our study had only moderate Se concentrations and so infiltration through these soils is not a major contributor to Se in groundwater. Our data showed that the relative abundance of Se species is dependent upon total Se in the sample. Samples with high total Se were dominated by organic Se, which is potentially mobile and could lead to environmental contamination. Organic matter plays a principal part in the accumulation and speciation of Se in different environmental media.

Acknowledgements

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