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The effects on soil/water/plant/animal systems by platinum group elements

Invited Review

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Abstract: Emissions of toxic substances such as oxides of carbon, nitrogen, sulphur, and, in addition, aromatic hydrocarbons, aldehydes and heavy metals are the most serious problem of road traffic affecting landscape. Platinum group elements (PGE), which are the main component of the catalyst, are one of the main sources of heavy metals in the environment. Here, we review the way by which emissions and forms of the emitted PGE end up in the environment especially to the soil-water-plant-animal system. The major points discussed are the following: 1) the main sources of PGE emission are automobile exhaust catalysts; 2) hospitals, where platinum is widely used to treat malignant neoplasm, and urban waste water belonging to other important sources of PGE in the environment; 3) soil is one of the most important components of the environment that may be contaminated with platinum metals; 4) phytotoxicity of PGE depends on the following conditions: the concentration of metals in the soil, time of exposure, the chemical form of metal, the chemical composition of exposed soil and plant species; 5) animals are also endangered by the increasing concentration of PGE in the environment. Moreover, we pay our attention to thiol-based mechanisms of how an organism protects itself against platinum group elements.

Keywords: *Platinum Group Elements • Plant • Soil • Phytochelatins • Metallothioneins*

1. Introduction

Increasing anthropogenic activities result in a burden on human health and the environment due to the emissions of toxic compounds. Road traffic is one of the fundamental anthropogenic activities that affect the landscape [1-3]. Emissions of toxic substances, which include oxides of carbon, nitrogen, sulphur, hydrocarbons, aldehydes and heavy metals (cadmium, zinc, chromium, iron, lead, copper, nickel, vanadium, manganese) are the most serious problems of road traffic. Emissions of pollutants associated with the road traffic have decreased significantly from 35 to 24% depending on the type of pollutant due to the extensive using of exhaust catalysts in the developed countries [4,5]. Platinum group elements (PGE; platinum, palladium, rhodium and ruthenium, less frequently iridium) in catalysts contribute to the reduction of pollutants in exhaust gases (Fig. 1). The disadvantage of using catalysts containing PGE is that there is considerable leakage of these elements into the environment. This amount can be enhanced by other anthropogenic industrial activities as jewellery and anticancer drugs based treatment protocols (Fig. 1). Thus, PGE contaminate the environment and consequently tend to bioaccumulate in organisms (Fig. 2).

Raining greatly contributes to the spread of contaminants in the environment. Analysing water that drains from roads and other places contaminated by PGE reveals interesting data about the presence

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of these rare metals and thus offers a way to monitor the bioavailability and accumulation of these ions in organisms. The results of many studies are now the basis for the assumption that these metals are able to accumulate in the tissues of organisms and thus may pose a serious threat [4,6-10]. Discussions about the need of systematic monitoring of the concentration of these elements in samples from the environment is growing due to the increase in concentration of PGE in the environment [4,11-18].

2. Physical and chemical properties of PGEs

Platinum, palladium and rhodium have similar physical and chemical properties, and tend to occur together in the same mineral deposits [19]. Table 1 summarizes the electron configuration, standard atomic weight, phase, density, melting point, boiling point, molar heat capacity, electronegativity and atomic radius for platinum, palladium and rhodium. In the state of pure metal, platinum is silvery-white, lustrous, ductile, and malleable [20]. Ductility of platinum is higher compared to gold, silver and copper, but gold is still more malleable than platinum [21,22]. Platinum does not oxidize at any temperature, although it is corroded by halogens, cyanides, sulphur, and caustic alkalis. Moreover, dissolves in hot aqua regia to form chloroplatinic acid, but it is insoluble in hydrochloric and nitric acid [6]. The metal has an excellent resistance to corrosion and high temperature and has stable electrical properties. The most common oxidation states of platinum are +2 and +4. The +1 and +3 oxidation states are less common, and are often stabilized by metal bonding in bimetallic (or polymetallic) species [22].

Palladium as a soft silver-white metal is the least dense and has the lowest melting point of the platinum group metals. It is ductile when annealed and cold-working greatly increases its strength and hardness. Palladium dissolves slowly in sulphuric, nitric and hydrochloric acid [23]. This metal also does not react with oxygen at normal temperatures, but under 800°C a layer of palladium(II) oxide (PdO) is formed. It lightly tarnishes in moist atmosphere containing sulphur [24]. Palladium primarily exists in the 0, +2, +4 oxidation states; the +4 oxidation state is comparatively rare.

Compared to palladium, rhodium is a hard, silvery, durable metal with a high reflectance. Rhodium metal does not normally form an oxide, even when heated [25]. Rhodium is able to absorb oxygen only at the melting point from the atmosphere, but the oxygen is released on solidification [26]. Rhodium has both a higher melting point and lower density than platinum. It is completely insoluble in nitric acid and dissolves slightly in aqua regia. The common oxidation state of rhodium is +3, but oxidation states from +0 to +6 are also observed [27].

3. Sources of platinum group elements

As mentioned above, the concentration of platinum metals in environmental samples such as soil, dust on roads, surface water, sediments and plants has significantly increased in the last decade [28-33]. The main sources of PGE are automotive emission catalysts, where their unique catalytic properties are used. Other specific properties of PGE are that they are particularly resistant to chemical corrosion in a wide temperature range. In addition, they have high melting points, high mechanical strength and good ductility. Based on these properties, PGE are also used in the glass, chemical, electrical, electronics and oil industry, as well as in the production of jewellery, in medicine to treat cancer and in dentistry for the preparation of dental fillings (Fig. 1). More information about the properties of PGE and their main sources can be found in the following papers [4,11,34-38].

3.1. Automobile exhaust catalysts

PGE are present in the catalysts in the form of very small particles. The small sizes of these metals are more prone to be affected by numerous factors that can increase their oxidation and thus the bioavailability

Table 1. Physical and chemical properties of platinum, palladium and rhodium [19-22].

PGE	Electron configuration	Standard atomic weight	Phase	Density [g cm^{-3}]	Melting point [°C]	Boiling point [°C]	Molar heat capacity [J mol^{-1} K^{-1}]	Electrone-gativity	Atomic radius [pm]
Platinum	[Xe] 4f^{14} 5d^{9} 6s^{1}	195.084	solid	21.450	1768.3	3825	25.86	2.28	139
Palladium	[Kr] 4d^{10}	106.420	solid	12.023	1554.9	2963	25.98	2.20	137
Rhodium	[Kr] 5s^{1} 4d^{8}	102.905	solid	12.410	1964.0	3695	24.98	2.28	134

PGE platinum group element

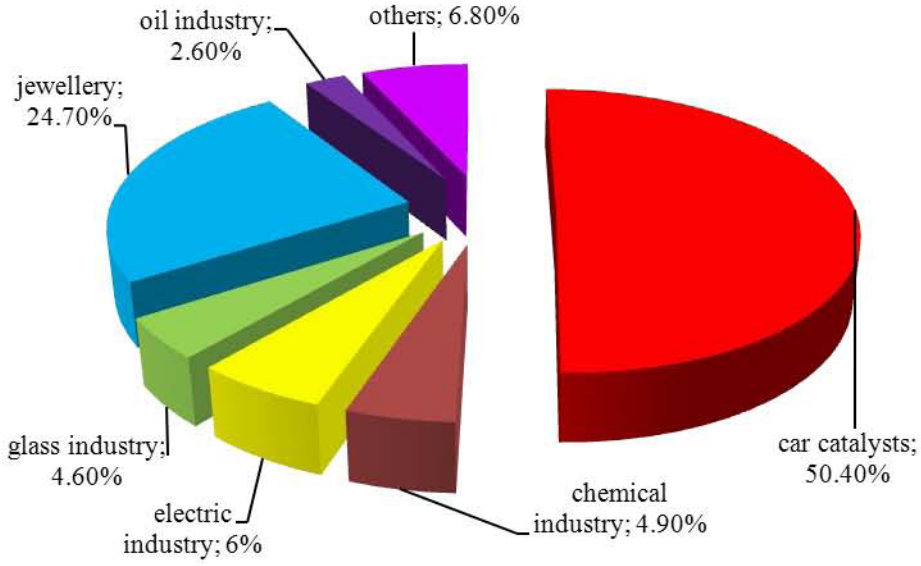

Figure 1. The main emission sources of platinum metals in Europe (2006). Automobile catalysts are responsible to the main percentage of emissions of platinum metals into the environment (50.40%). Other sources are by-products in jewellery (24.7%), electric industry (6%), chemical industry (4.9%), glass industry (4.6%) and oil industry (2.6%).

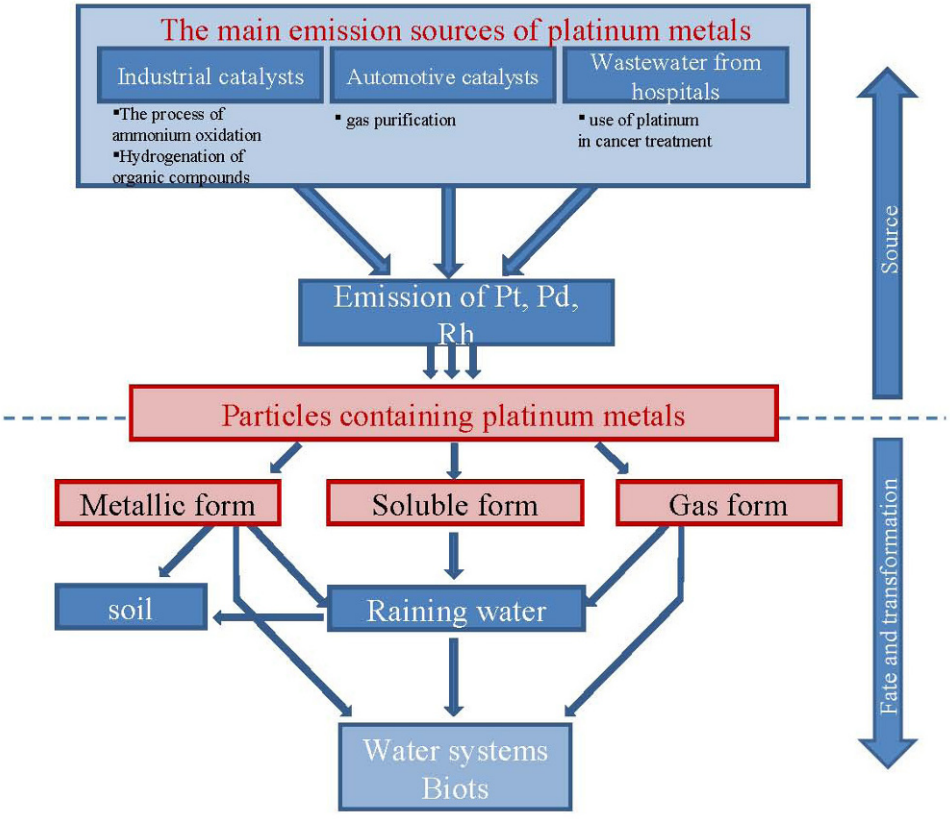

Figure 2. The main sources of emissions of platinum metals are primarily industrial and automotive catalysts as well as wastewater from healthcare, where they are used for platinum anticancer therapy. Industrial catalysts are used for various oxidation processes and organic compounds hydrogenation, while automotive catalysts are used to clean exhaust gases. Platinum metals particles enter into the environment. The nature of the particles determines the next cycle. Metal form usually goes directly into the soil, but can be dissolved in small concentrations of acid rain. Soluble form is getting into the water cycle and can be retained in the soil. Similarly, it is in gaseous form. Therefore, all the particles get to the water systems and biota.

Table 2. Table providing PGE concentrations measured in road dust, soil and water from various locations from 2007 to 2012. The locations are divided according to PGE, source and found concentration.

PGE	Source	Location	Concentration		Reference
Platinum	Road dust	Seoul, Korea	0.7-221	ng g^{-1}	[133]
		Hyderabad, India	1.5-43	ng g^{-1}	[134]
		Greece	306.4	ng g^{-1}	[135]
	Soil	Seoul, Korea	3.8-444	ng g^{-1}	[133]
		Italy	4.9-20	ng g^{-1}	[136]
		Germany	50.4-261	ng g^{-1}	[137]
		Hong Kong, China	160	ng g^{-1}	[138]
		Greece	225	ng g^{-1}	[135]
	Water	Ghana	0.004-0.012	μg L^{-1}	[139]
	Antarctic water	East Antarctica	4.7-76	fg g^{-1}	[140]
Palladium	Road dust	Hyderabad, India	1.2-58	ng g^{-1}	[134]
		Greece	18.2	ng g^{-1}	[135]
	Soil	Greece	4.0	ng g^{-1}	[135]
		Germany	43.3-124	ng g^{-1}	[137]
	Water	Ghana	0.003-0.019	μg L^{-1}	[139]
Rhodium	Road dust	Hyderabad, India	0.2-14.2	ng g^{-1}	[134]
		Greece	64.6	ng g^{-1}	[135]
	Soil	Germany	10.7- 38.9	ng g^{-1}	[137]
		Hong Kong, China	34.5	ng g^{-1}	[138]
		Greece	49.5	ng g^{-1}	[135]
	Water	Ghana	0.001-0.002	μg L^{-1}	[139]
	Antarctic water	East Antarctica	0.12-8.8	fg g^{-1}	[140]

[39-41]. Moreover, the content of bioavailable forms of these metals in exhaust gases increases with the period and the intensity of a catalyst used [37,39,41,42]. PGE emission rate is also influenced by the speed of the car, engine type and type of fuel additive [8,33]. Emissions can be also enhanced by adverse operating conditions (ignition, excessive heat), which can even destroy the catalyst [43]. Platinum metals are mostly emitted in compounds with halogens derived from fuel additives or in the form of carbonyl complexes [39]. The bioavailability of active form of platinum is given by oxidation state and/or ligand. Therefore, it was assumed that metals emitted in the oxidation state "0" do not enter in biogeochemical cycles due to their chemical inertness. However, the results of studies on the solubility of selected chemical forms of PGE have shown that many factors could increase their bioavailability. Significant increases in PGE bioavailability can be observed in the presence of some organic compounds such as humic acids, fulvic acids, acetates, or metabolic products of fungi, bacteria and plants [5].

3.2. Wastewater from hospitals

Hospitals and urban waste waters belong to the other important sources of PGE in the environment. In connection with the use of various derivatives of platinum cytostatics (cisplatin [cis-platinum diaminedichloro (II)] and carboplatin [diamine (1,1-cyclobutanedicarboxylato) platinum (II)]), which are widely used to treat malignant neoplasms [44], these metals can migrate into drainage and subsequently into the wastewater. This was in the paper monitoring the concentration of platinum in the effluent of 5 hospitals in Austria, Belgium, Germany, Italy and the Netherlands, which were specialized in the treatment of neoplasms [36,45]. It was found that the majority of platinum-based drugs (70%) was excreted by urine and entered to wastewater of the hospitals. The concentration of platinum reached the concentration <10 ng L^{-1} in the effluent of the Belgian and Italian hospitals, about 3.5 ng L^{-1} in the German and Austrian hospitals. In all cases the platinum level did not exceed 10 ng L^{-1}. In comparison with other sources of PGE, such as automotive catalysts, wastewaters from hospitals is still a negligible source.

4. Transport of platinum group elements in the environment

There is limited data on the assessment of PGE bioavailability or on their fate in the environment. In spite of the fact that there is increasing attention paid to this topic, very low content of these metals in samples obtained from the environment and due to

the difficulties of their detection by modern analytical instruments are the main obstacles. Based on some expert opinions, emitted PGE, whether in the form of metals or compounds, shows only a low toxic potential. However, the mobile part of the emitted metals, as well as the part that undergoes conversion to soluble forms represents a serious threat to both plants and animals [4,11].

4.1. Soil

Soil is one of the most important components of the environment that may be contaminated with PGE [5,6,46,47]. Anthropogenic emissions of PGE lead to a significant increase of their concentration in soil and dust, especially near roads (Fig. 2). This process can be very well documented by comparing the average concentration of platinum in samples of different soil types: soil without anthropogenic interference - 0.14 mg kg^{-1}, agricultural soil - 1.1 mg kg^{-1}, and soil from areas located near the road - 20.9 mg kg^{-1} [48,49]. Distribution of PGE in the soil near the main motorways is associated with traffic intensity and road conditions [50-53]. The results of determination of PGE in soil samples nearby the road showed that the concentration of platinum, palladium and rhodium decreased with the increasing distance of the sampling spot from the road, while the highest concentration found in the range of two meters from the road [54]. PGE content in the soil also decreases with the sampling depth [29,55], which may be associated with a slow migration of PGE in the soil [48]. Physico-chemical properties of soil play key role in bioavailability of PGE, because the processes of chemical transformations of PGE forming easily or not accessible compounds is fully dependent on the environment and influences further transport of these metals [5]. PGE mobility in soil depends on many factors such as pH, redox potential and soil salinity. Mechanisms of PGE transformation and their relation with the biologically available forms are usually associated with chemical oxidation, complex-forming reaction with organic ligands present in the soil, and biochemical transformations with the help of bacteria. Moreover, it has been described that some naturally occurring organic ligands such as L-methionine have an influence on the bioavailability of platinum and palladium in soil [56-60]. The presence of humic acids in the soil leads to immobilization of platinum salts in the form of poorly soluble organic complexes. On the other hand, organic compounds, such as EDTA or thiourea, increase soil bioavailability of platinum and palladium [61]. Biomethylation of these elements by soil bacteria is one of the most recent to increase the bioavailability of PGE [62-65].

4.2. Water and ecosystems

Several recent studies have reported increasing concentration of PGE in different parts of the aquatic ecosystem (rain, drinking and ground water, sea water, river and sea sediments, sewage sludge, *etc.*). Increasing of PGE concentration in water systems can be most likely related to PGE coming from the exhaust gases, which also supports the evidence of the mobility of platinum and palladium from samples of sediment [66]. The highest content of these metals was determined in the immediate vicinity of roads, which reached values of 50 ng g^{-1}, while the lowest values were observed in sediment rocks. The content of PGE in river flows generally ranged from 0.4 to 10.8 ng g^{-1} [4]. Even though the concentration of PGE in comparison with other anthropogenic environmental matrices is relatively low, we can expect a large impact of these pollutants on aquatic animals due their ability to bioaccumulate. For an idea of how much platinum and palladium from anthropogenic emission enters nature through the waste water, samples from sewage and sewage sludge in Boston were analysed. Boston sewage is a mixture of household and industrial waste as well as road run-off, which is normally routed through the sewage treatment system. The concentration of platinum and palladium in sludge were close to the results measured in New York and some European cities [67], *i.e.*, platinum (<10 to 1,070 µg kg^{-1}) and palladium values (38 to 4,700 µg kg^{-1}). Helmers *et al.*, which also dealt with the content of PGEs in waste water in Germany, came to the conclusion that such high a concentration of platinum and palladium in these matrices cannot be given only by increasing effects of car traffic and thus they concluded that the PGEs also have origins in other sources, especially in the chemical industry that can easily release dissolved or easily soluble compounds to the environment [68].

4.3. Influence of PEG an organism

4.3.1. Plants

Plants are the first member of the food chain. Studying the ability of plants to uptake PGE from the soil is very important in the protection of human health [69-71]. The intake of heavy metals in the soil is mainly due to the presence of complexing agents (organic acids), which the plant is able to secrete into the soil. Available literature suggest that PGE (mainly palladium) are received from the soil through the roots, and then PGE binds to the biologically active substances rich in sulphur [60,65,72-74]. PGE accumulation process takes place mainly in the vegetative parts of plants and decreases in the following order: root> stem> leaves. Furthermore, it was observed thatlarger quantities of PGE was accumulated in surface tissues

of underground plant parts [43]. Plant species also play a key role in the ability to uptake PGE from soil. Results of the analysis of plants (watercress, spinach, nettle) grown in the soil collected nearby the highway show that palladium is characterized by the highest bioavailability, followed by rhodium and platinum [43,65]. Based on the bioaccumulation coefficient, which is defined as the ratio of element concentration in plant material and its concentration in the soil close to plants, it is possible to include PGE among the poorly to moderately available metals for plants, like copper and nickel [18,43]. Chemical forms also affect the availability of PGE of plants. In the case of platinum at least biologically available metal platinum, the bioavailability is increased in the case of $PtCl_4$ and is the highest for the complex $Pt(NH_3)_4(NO_3)_2$ [5]. Not only land plants, but also aquatic plants are able to accumulate PGE. Experimental studies with water hyacinths showed that the PGE content increased with increasing concentrations of these metals in the water. Like other plants, there PGE toxicity depends on its chemical form, and increases in the following order Rh(III) << Os(IV) ~ Pt(IV) < Ir(III) ~ Ru(II) ~ Ru(III) < Pd(II) ~ Pt(II) [75]. The presence of PGE is observed mainly in plants growing in areas near streets and highways with traffic. Results of studies conducted on samples of moss [76] and grass [77] collected a near the road supported that catalysts are one of the main sources of emissions into the environment of platinum. The study showed a relationship between the numbers of cars equipped with catalytic converters and increased concentrations of platinum in plant material. It is likely that high concentrations of PGE in selected samples of plants may be due to the ability of plants to uptake metals by their root system from the soil or water, but also from their surface. The mechanism of this type of uptake is still unknown and the controversial results have being presented [18,54,78]. Based on information available, we can assume that phytotoxicity of PGE depends on the following conditions: the concentration of metals in the soil, exposure time, chemical form of the metal, the chemical composition of the exposed soil and plant species.

4.3.2 Animals

In spite of the great interest in the studying of PGEs, less is known regarding the potential impacts of environmental exposures to PGE, mainly data on the subclinical effects of chronic low dose exposures are not available. This is especially relevant regarding attempts to assess the effects of exposures to low concentrations of PGE that are being continuously emitted into the environment. Due to intensive using of platinum and platinum-containing substances in chemotherapy, there are numerous studies. On the other hand, toxicity of Pd and Rh and the behaviour of particle bound PGE emitted from catalytic converters is not known. The acute toxic effects of PGE are dependent on metal speciation, where the best soluble are the most toxic. Hexachloroplatinic acid, for instance, is highly nephrotoxic in rats [1], while metallic Pt appears to be only mildly toxic. Several Pt compounds have been found to be mutagenic in bacterial systems, especially the commonly used anti-neoplastic agent cisplatin. Pd salts appear to have a lower genotoxicity in bacteria and mammalian cells compared to Pt [2,3], which was confirmed on human lymphocytes [79]. In rats exposed to various concentrations of Pt salts per gavage over a 4 week period, Pt was found to cause DNA damage, shrinkage of the glomeruli in the kidney and induce the development of eosinophil inclusion bodies in the adrenal glands [80]. There was also found enhanced immune response to Pt, as indicated by a hyperactivity of the lymphocytes.

Although very little is known about the autoimmune effects of PGE exposure, metal ions which possess a high redox potential such as Pt(IV), do have the ability to denature proteins by oxidizing the sulphur-containing amino-acid side chains. Further, metal ions such as Pt(II) may also form coordination complexes with proteins, which could also serve to interfere with the processing and presentation of self-proteins and -peptides, resulting in activation of autoreactive T cells. There was published epidemiological study demonstrating an association between PGE exposures and rates of autoimmune diseases in humans, in which Pd was determined in urinary samples from 367 participants [81]. It was found that those who reported to have autoimmune diseases such as Type 1 diabetes and hypo-/hyperthyroidism had elevated levels of Pd.

Some *in vitro* studies suggest that Pd(II), Pt(II) and Rh(III) complexes are able to inhibit a variety of cellular functions, which can be attributed to their capacity to form strong complexes with various organic and inorganic ligands. There was also demonstrated that PGE can affect cellular fluidity, membrane integrity and permeability and interfere with protein synthesis [82,83], inhibit mitochondrial enzyme production and alter the structure of the mitochondrial lipid bilayer [84]. Frazzoli *et al*. [85] investigated the potential of $PdCl_2$, $PtCl_2$ and RhCl3 to interfere with aerobic respiration at a cellular level using suspended yeast cells (Saccharomyes cerevisiae). Metal concentrations were found to closely correlate with acute respiration inhibition. The lowest experimental doses found to induce an adverse effect were 161 ng g^{-1} for Pd, 120 ng g^{-1} for Pt and 60 ng g^{-1} for Rh. Interestingly, they found that Rh was more toxic (*i.e*., EC50%, steepness of curve) than Pd

and Pt used human bronchial epithelial cells (BEAS-2B) to examine the effects of PGE exposure on cellular viability and their capacity to induce the release of reactive oxygen species as an indicator of oxidative stress [86]. Inorganic PGE salts were used ($Pt(NO_3)_2$, $PtCl_4$, $PdSO_4$, $RhCl_3$) and compared to the effects of Cd, Cr and Ag salts. The LC50 for Pt(IV) was 0.05 mmol L^{-1} and Pt(II) and Pd(II) had a LC50 of 0.4 nM, which is similar to heavy metal species such as Cd(II) and Cr(IV). Rh demonstrated a low toxic potential. Pt(II) and Pt(IV) were found to lead to an increase in the production of reactive oxygen species, while no effect was observed for Pd(II) and Rh(III). Similar to other metals such as Hg, Pb and Cd, PGEs have also been found to be among the most potent inducers of metallothionein (MT) protein synthesis, a commonly used indicator of metal toxicity which appears to play a role in detoxification [87-93].

There is also interest in the studying of the effects of PGEs on foetus. An earlier study on rats exposed to Pd and Pt salts through various routes, Pd and Pt were shown to be capable of passing through the placental barrier in pregnant rats [94]. Gagnon and Patel [95] demonstrated that Pt accumulated at a higher rate in the brain of chicken embryos compared to the liver, which is likely due to the absence of a fully developed blood-brain barrier. Induction of MT synthesis in the brain and liver of chick embryos exposed to PGE was also observed. Given more recent evidence regarding an association between environmental exposures to metals during this highly vulnerable early stage of development and a variety of later health effects ranging from attention deficit hyperactivity disorder (ADHD) to Alzheimer's [96], there should be paid an attention to this.

One major concern regarding PGE exposures is the sensitization potential of these metals, especially that of their salts [4,97]. Pt salts have been particularly associated with an increased incidence of asthma, rhinoconjunctivitis and dermatitis and urticaria among workers occupationally exposed to these metals such as those in catalyst production and refineries [98-100]. In the case of Pd, patch testing in various clinics have shown that as many as 13% of tested individuals reacted positively with this metal [101]. The respiratory sensitization potential of environmental exposures to PGE among the general population, a primary toxic endpoint of concern, is however unclear. A study conducted in Nordrhein-Westfalen, Germany, showed that ambient Pt levels significantly correlated with the number of neutrophils and epithelial cells in the nasal lavage fluid of children, which have been established to be good biomarkers for upper airway inflammation [102]. Although environmental levels of PGE are generally much lower than that found among those who are occupationally exposed, such studies indicate that ambient exposures to these metals may elicit effects on a subclinical level that are not readily detected among the general population [78].

4.3.3. Clinical trials

Cisplatin is still one of the most important and used drugs in chemotherapy treatment protocols of various malignancies [103]. Unfortunately its continued use is greatly limited by severe dose limiting side effects and intrinsic or acquired drug resistance. Since discovery of cisplatin hundreds of platinum(II) and platinum(IV) complexes have been synthesized and evaluated as anticancer agents over past 40 years, but carboplatin and oxaliplatin only have entered clinical trials almost worldwide, and another three (nedaplatin, lobaplatin and heptaplatin) gaining approval in individual nations. Currently there are four drugs in the various phases of clinical trial (satraplatin, picoplatin, Lipoplatin (TM) and ProLindac (TM)). No new small molecule platinum drug has entered clinical trials since 1999, which is representative of a shift in focus away from drug design and towards drug delivery in the last decade [104]. The antitumor activity of the inorganic complex cisplatin led to the development of other types of non-organic cytostatic drugs. Numerous platinum other platinum and non-platinum metal compounds have been shown to be effective against animal model tumours as well as tumours in man. However, the introduction of novel transition metal agents in clinical treatment is exceptionally slow. So far, Ru(II) and Ru(III) complexes have shown very promising properties while the Ru(III) compound, [ImH] [trans-$Cl_4(Me_2SO)(Im)Ru(III)$] (Im=imidazole, NAMI-A), is the first ruthenium compound that successfully entered phase I clinical trials. There have been also tested rhodium based compounds, but found to be less effective as anticancer agents mainly due to their toxic effects. Dimeric μ-Acetato dimers of Rh(II) as well as monomeric square planar Rh(I) and octahedral Rh(III) complexes have shown interesting antitumor properties [105].

To evaluate the effect of palladium based therapies, some clinical trials have been done. Radioactive Pd-103 seeds have become available for plaque brachy-therapy, and computer-aided simulations have compared the intraocular dose distribution of Pd-103 *versus* iodine 125 (I-125) plaques in patients with uveal melanoma. The use of the lower-energy radionuclide Pd-103 increased the radiation to the tumours and decreases irradiation of most normal ocular structures. The authors have begun a phase I clinical trial evaluating the effect of Pd-103 ophthalmic plaque radiotherapy on intraocular tumours. Uveal melanoma was diagnosed, and the patients

were found to be negative for metastatic disease. All patients were given one Pd-103 radioactive plaque treatment, and six patients also were given adjuvant microwave hyperthermia. Palladium 103 ophthalmic plaque radiotherapy was used to treat 23 patients with uveal melanoma. Patients were followed for up to 27 months (mean, 13.5 months). One eye was enucleated for progressive tumour enlargement (4 months after treatment). One patient died (of metastatic melanoma). Eight patients have lost greater than two lines of visual acuity, one has gained more than two lines. Fifteen patients (65%) were within two lines or had better than their preoperative visual acuity. Relating to the effect of treatment on visual acuity, 15 (65%) tumours were located equal to or less than 2 mm from the fovea. Palladium 103 ophthalmic plaque radiotherapy was noted to control the growth of uveal melanomas. Compared with other forms of plaque radiotherapy at this follow-up interval, the authors have noted no new complications, no difference in local control, and/or changes in tumour response to treatment. More long-term follow-up will be required to demonstrate differences between I-125 and Pd-103 ophthalmic plaque brachytherapy [104].

5. Biologically important thiols

Metal ions have been acquired in the course of evolution because of their chemical properties such as redox–activity under physiological conditions (Cu, Fe) or Lewis acid strength (Zn) [106,107]. The same properties that make transition metal ions indispensable for life, however, are also the reason why they can easily be toxic when present in excess. The main threat lies in their ability to produce reactive oxygen species (ROS) [108]. Unfortunately, toxic metals such as cadmium, lead, mercury and above presented PGE as well as the essential ones can also produce ROS [109,110], see in Fig. 3.

A thiol is an organo-sulphur compound that contains a carbon-bonded sulfhydryl (–C–SH). As the functional group of the amino acid cysteine (Fig. 4A), the thiol group plays an important role in biology. When the thiol groups of two cysteine residues (as in monomers or constituent units) are brought near each other in the course of protein folding, an oxidation reaction can generate a cystine unit with a disulphide bond (-S-S-). Disulphide bonds can contribute to a protein's tertiary structure if the cysteines are part of the same peptide chain, or contribute to the quaternary structure of multi-unit proteins by forming fairly strong covalent bonds between different peptide chains. In addition, the sulfhydryl group is highly reactive and is often found conjugated to other molecules, mainly to heavy metal ions [111]. It is not surprising that biologically active molecules rich in –SH moiety are responsible for maintaining homeostasis of metal ions and/or their detoxifying (Fig. 5).

5.1 Reduced glutathione

Reduced glutathione (GSH, Fig. 4B), a ubiquitous tripeptide thiol, is a vital intra- and extra-cellular protective antioxidant, which plays a number of key or crucial roles in the control of signalling processes, detoxifying of some xenobiotics and heavy metals as well as other functions. Glutathione is found almost exclusively in its reduced form; since the enzyme, which reverts it from its oxidized form (GSSG) called glutathione reductase, is constitutively active and inducible upon oxidative stress. The glutathione-ascorbate cycle is a metabolic pathway that detoxifies hydrogen peroxide (H2O2), which is a reactive oxygen species that is produced as a waste product in metabolism. The cycle involves the antioxidant metabolites: ascorbate, glutathione and NADPH and the enzymes linking these metabolites. In the first step of this pathway, H2O2 is reduced to water by ascorbate peroxidase using ascorbate as the electron donor. The oxidized ascorbate (monodehydroascorbate) is regenerated by monodehydroascorbate reductase. However, monodehydroascorbate is a radical and if not rapidly reduced it disproportionates into ascorbate and dehydroascorbate. Dehydroascorbate is reduced to ascorbate by dehydroascorbate reductase at the expense of GSH, yielding oxidized glutathione (GSSG). Finally GSSG is reduced by glutathione reductase using NADPH as electron donor. The reduction of dehydroascorbate may be non-enzymatic or catalysed by proteins with dehydroascorbate reductase activity. Under normal conditions, the GSH redox couple is well known to be present in mammalian cells in the concentration range of 1–10 mM. In a resting cell, the molar GSH:GSSG ratio exceeds 100:1, whereas in various models of oxidative stress, this ratio has been reported to decrease to values between 10:1 and even 1:1. In connection with this, many fundamental events of cell regulation such as protein phosphorylation and binding of transcription factors to consensus sites on DNA are driven by physiological oxidant-antioxidant homeostasis, especially by thiol-disulphide balance. Therefore endogenous glutathione and thioredoxin systems and may be considered to be effective regulators of redox-sensitive gene expression [112]. GSH can interact well with platinum as it was shown

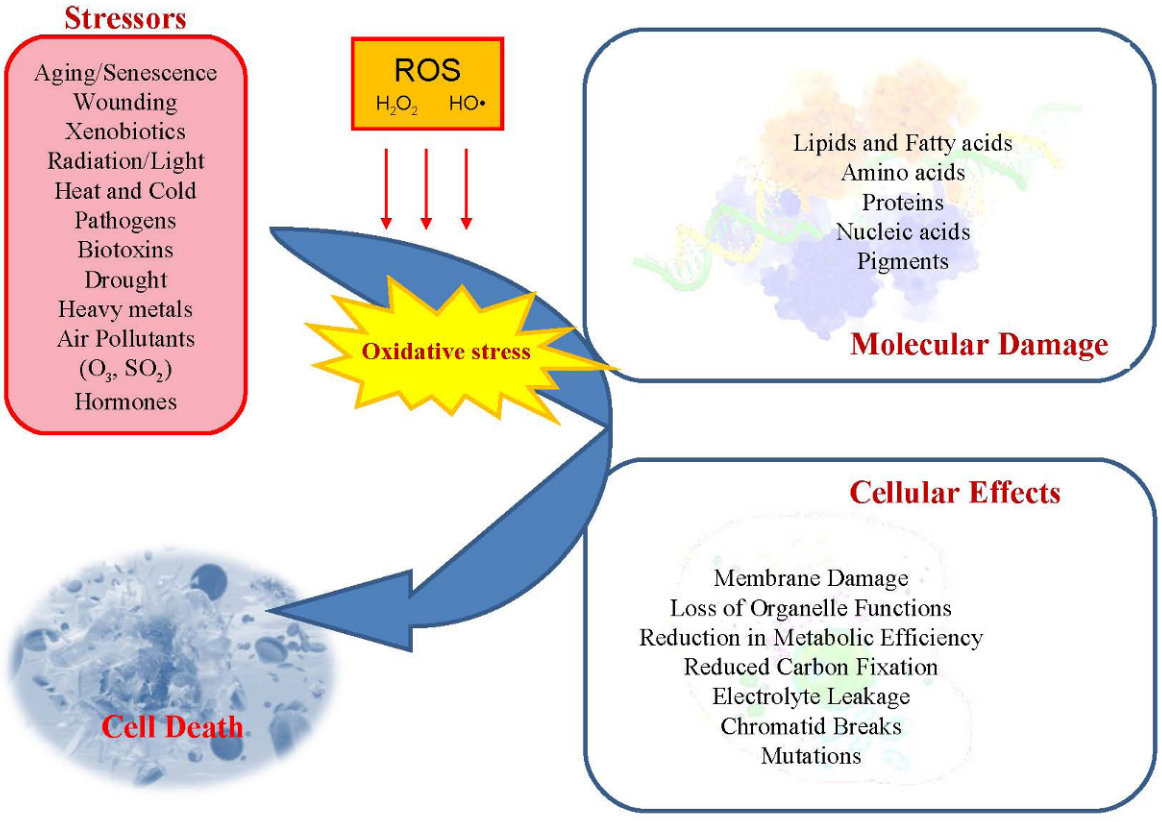

Figure 3. Scheme showing some of the initiators (stressors) of reactive oxygen species (ROS) and the biological consequences leading to a variety of physiological dysfunctions that can lead to cell death.

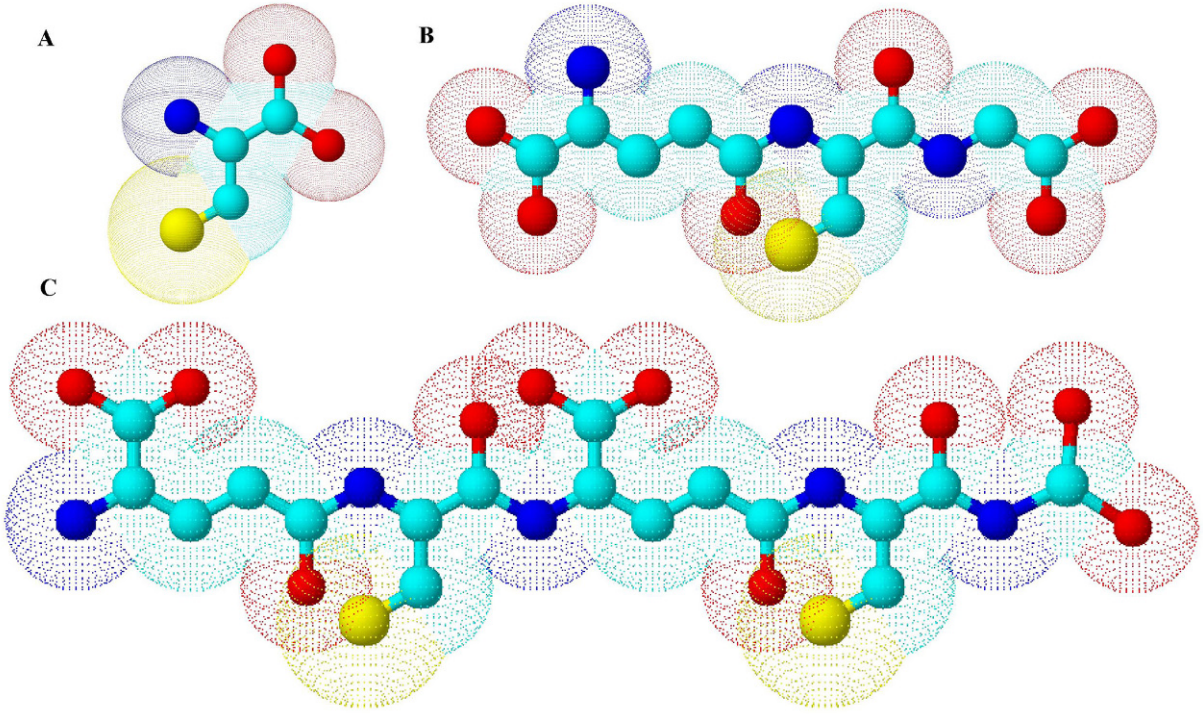

Figure 4. Model of (A) cysteine, (B) reduced glutathione and (C) phytochelatin2.

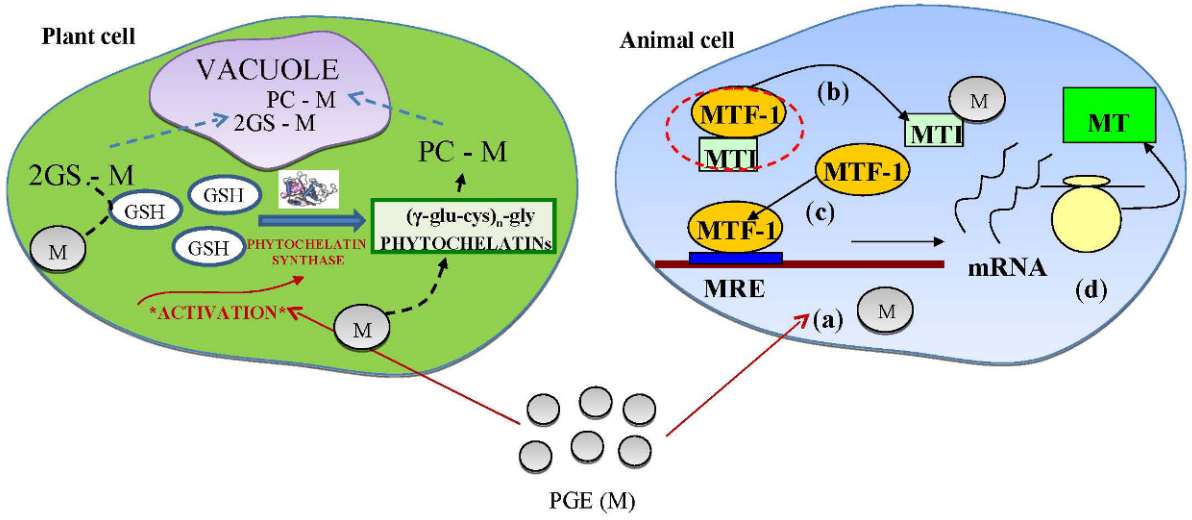

Figure 5. *Plant cell.* Glutathione serves as a precursor of phytochelatins, which are composed of two or more repeating gamma-glutamylcysteine units with a terminal glycine residue; (gamma-glutamylcysteine)$_n$-gly, where n = 2 to 11. The enzyme responsible for the synthesis of these peptides is known as phytochelatin synthase (glutathione gamma-glutamylcysteinyltransferase or gamma-glutamylcysteine dipeptidyl transpeptidase), which is a constitutive enzyme that is activated by cadmium and other metal ions. *Animal cell.* Metal ions enter through a cytoplasmic membrane using ionic channels or special transporters (a). After the entering the cytoplasm, the ions interact with complex of metal-regulatory transcription factor-1 (MTF-1) and metal synthesis inhibitor (MTI) (b). The ions bind to MTI. Due to the binding of the ions to MTI, MTF-1 is released and can bind to a regulatory sequence of DNA called metal responsive element (MRE) (c). Further, the gene responsible for synthesis of metallothioneins is transcribed. The synthesized mRNA molecule is translated into MT (d). MT binds to the heavy metal ion.

by Zitka *et al.* [113]. On the other hand, there have been published numerous papers on the relation of glutathione and platinum based drugs [114,115] but environment aspect have not been discussed [116].

5.2. Phytochelatins

Moreover GSH can be used for synthesis of phytochelatins (a basic formula (γ-Glu-Cys)n-Gly (n = 2 to 11), molecule of PC2 is shown in Fig. 4C) participating in the detoxification of heavy metals at plants, because they have the ability to bind heavy metal ions *via* SH groups of cysteine units and consequently transport them to vacuole to detoxify them. The synthesis PC itself involves the transpeptidation of the γ-Glu-Cys moiety of GSH onto initially a second GSH molecule to form PC2 or, in later stages of the incubation, onto a PC molecule to produce an n + 1 oligomer. The reaction is catalysed by γ-Glu-Cys dipeptidyl transpeptidase (EC 2.3.2.15), which has been called as phytochelatin synthase [117,118]. *In vitro* the purified enzyme was active only in the presence of metal ions. Cadmium was the best activator of phytochelatin synthase followed by Ag, Bi, Pb, Zn, Cu, Hg, and Au cations [119]. Supalkova *et al.* found that marked increase in thiol concentration detected is associated with defence reaction of the plant against stress caused by cisplatin [120]. Lesniewska *et al.* studied bioaccumulation of Pt, Pd and Rh by grass grown hydroponically with nutrient solutions containing these ions. The highest bioaccumulation factors were obtained for Pd and Rh in roots and for Pt in leaves. The obtained results showed that most of the studied metals were accumulated in roots, and only a small fraction was really metabolized and transported to leaves. The authors also concluded that the presence of Ca, Cu, S and C in the same fractions as Pt, Pd and Rh may indicate the interaction of PGEs with phytochelatins and carbohydrates [121].

5.3. Metallothionein

Metallothioneins (MTs, Fig. 5) were discovered by Margoshes and Valee in 1957 as newly identified proteins isolated from a horse renal cortex tissue. These proteins occur in whole animal kingdom with high degree of homology. Similar proteins are expressed by bacteria, fungi and even plants. MTs are low molecular mass (from 2 to 16 kDa) proteins with unique abundance of cysteine residues (more than 30% from all aminoacids). Another interesting structural property is the lack of aromatic amino acids. However - as discovered recently – there is an exception: a group of certain yeast and bacterial species occasionally containing histidine. MTs are single-chain proteins with amino acid number oscillating between app. 20 and more than 100 residues according to organisms. Almost one third of this number is cysteine occurring in conserved sequences *cys-**x**-cys*, *cys-**x**-**y**-cys* a *cys-cys* where **x** and **y** represent other amino acids. Divalent metal ions bonded to sulfhydryl groups of cysteines are

creating tetraedric configuration of thiolate clusters. MT exhibits the highest affinity for Cu^{+} (stability constant 10^{17} – 10^{19}), followed by Cd^{2+} (10^{15} – 10^{17}) and Zn^{2+} (10^{11} – 10^{14}); however it is not capable of binding Cu^{2+}. Generally, 18 metal ions suitable to be bonded by MT are known but only Cu^{+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Ag^{+} and Bi^{2+} can replace Zn^{2+} in MT structure. Binding capacity of MT is 7 and 12 atoms for divalent and monovalent ions, respectively [122]. MT`s tertiary structure consists of two domains: more stable α (C-terminal), containing 4 ion binding sites, and β (N-terminal) capable to incorporate 3 ions [123]. The scheme of MT detoxifying system is shown in Fig. 5.

Expression of MTs is started by the binding of metal regulatory-transcription factor – 1 (MTF-1) to the regulative region of MTs gene called metal responsive element (MRE). Transcription of MTs through the MRE may be initiated by several metal ions (Zn, Cd, Cu, Hg, Pb, Au and Bi) however only Zn can activate MTF-1. Moreover, MRE is capable to interact with many proteins, which can regulate MT expression. Induction of MT expression by chemicals producing free radicals as well as various organic solvents has been shown. It has been found that the expression reaches the highest levels in the late G1 phase and during onset of the S phase. Nowadays the attention is focused on MT's role in cancerogenesis and on its relation to cancer cell cycle [124]. There have been several papers published dealing with interactions of platinum based cytostatics with MT due to possible role of these proteins in resistance against these metal drugs [92,125-129]. Attention has also been paid to environmental problems and MT has been determined in several species [95,130-132].

6. Conclusion

Distribution of PGE directly corresponds with the purposeful use of land (city vs. village) and a number of other anthropogenic activities such as sewage discharges (industrial, from health care) contribute to their increased concentration in the environment. A clearly identifiable source still remains catalysts for exhaust gases. Other factors contribute merely to the ratio of PGE concentration. Despite the significant increase in publications on topics of PGE there is still only a limited amount of information aimed at evaluating their bioavailability, methods and mechanisms of uptake and their fate in the environment. Therefore further research on these topics is much needed.

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