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NOTE

Heavy metals and oxidative stress: where do we go from here?

Juliana A. Azevedo[1], Ricardo A. Azevedo[2]*

[1] Universidade Estadual Paulista "Julio de Mesquita Filho", Rio Claro 13500-230, SP, Brazil.
[2] Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba 13418-900, SP, Brazil.
* Corresponding author: Ricardo A. Azevedo, E-mail: raazeved@esalq.usp.br

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ABSTRACT
In this perspective article, we briefly describe what researchers working on oxidative stress induced by heavy metals have accomplished and what would likely be the next stage in the research on this subject. Many papers have been published focusing on the physiological aspects; many of them may look quite similar. We propose that the emerging new "omics" techniques that are being widely employed in other areas should also be adopted for the study of heavy metal-induced oxidative stress and the general effect on plant metabolism. We believe that it is also necessary to integrate different areas, such as biology, phytogeography, soil sciences and even anthropology to get a more dynamic view of the problem. Such changes might add significant new information to our knowledge on the subject.
Key Words: *heavy metals, metabolomics; oxidative stress; proteomics*.

INTRODUCTION

Heavy metals are included in the main category of environmental pollutants as they can remain in the environment for long periods; their accumulation is potentially hazardous to humans, animals and plants (Benavides et al., 2005; Gratão et al., 2005a). We have witnessed in the last few decades a dramatic, worrisome increase in contamination of the environment, including soil, air and water. It would appear that humans are the only ones to blame, because anthropogenic activities are the main source of the pollution that is causing the contamination (Gratão et al., 2005a). Moreover, there are numerous reports indicating that accidents, such as the contamination of the soil in mining areas, spills of toxic metals into rivers and eventual contamination of water, among other possible events, have been responsible for the deterioration of the environment and have affected peoples' lives in several ways. It is important to mention that such disasters have not solely been observed in developing countries, but throughout the world.

It is quite obvious from the studies carried out along the years that heavy metals have adverse effects on plants and their productivity, although some metals are essential for plant growth in small quantities (Gomes-Junior et al., 2006). Furthermore, these metals can enter the plant system, accumulate and later may enter the food chain and cause harm to humans and animals (Vitória et al., 2001). The accumulation of metals in plants is particularly important because some species have been characterized as hyperaccumulators. They may be used, as along with specifically designed transgenic plants, in phytoremediation (Cherian and Oliveira, 2005; Eapen and D'Souza, 2005; Gratão et al., 2005b). Moreover, the use of bioremediation techniques (Lynch and Moffat, 2005) has been replacing whenever possible the traditional engineering approaches.

Such a situation has led to investigations on a wide range of aspects related to heavy metals. For instance, there has been intensive research on metals in soil related to plant nutrition, general effects on plant metabolism, tolerance-susceptibility, and environmental effects as well as on how contaminated areas can be reclaimed (Gratão et al., 2005a; Pilon-Smits, 2005; Taulavuori et al., 2005). This can be easily verified by searching websites, such as the *ISI Web of Knowledge* (http://portal.isiknowledge.com/), *SCOPUS* (http://www.scopus.com/scopus/home.url/) and *Google Scholar* (http://scholar.google.com/), among others.

As a matter of fact, the number of papers published on certain aspects of heavy metal research is large. The scope of some journals is wide-ranging for this particular field; some journals publish some papers specifically on certain aspects related to heavy metals and other contaminants, e.g., the I*nternational Journal of Phytoremediation, Environmental Pollution, Chemosphere, Science of the Total Environment*, among others.

It is well known that any pollutant, heavy metals in particular, has the potential to cause damage to plants, which, in turn, respond to the stress to survive. The plant defense system is varied and has been a major focus of attention in recent years. The late 1990s and the first few years of the 21st century witnessed a boom in the number of papers published in which the antioxidant defense system was investigated in detail in response to heavy metal-induced stress in plant tissues. For instance, a quick perusal of the *ISI Web of Knowledge* revealed that a huge increase in the number of papers related to heavy metal-induced oxidative stress in plants occurred from the late 1990s onwards. It is quite difficult to establish exactly which specific aspects have received most attention and it is essentially impossible to verify precisely the number of papers produced. Therefore, we used some very general keyword combinations, including "heavy metals," "oxidative stress," "plants," with some other more specific keywords, such as "glutathione," "catalase," "cadmium," "phytochelatins," among others, to detect any trend in the *ISI Web of Knowledge*. Results of some of these searches are presented in Table 1. Independent of a combination of keywords tested, tremendous increases in the number of papers published particularly in more recent years were observed. When the "heavy metals AND plants" combination was used, 14 papers published in 1990, 190 in 2000, and about 408 in 2005 were identified. Interestingly, when the keywords "oxidative stress" and "heavy metals" were used, a total of 16 papers published between 1990 and 1999 and 95 papers published between 2000 and 2005 were found (Table 1). When more specific keywords were used, e.g., "cadmium AND glutathione AND plants," zero papers in 1990, 18 in 1998, and 59 in 2005 were found (Table 1). Similar results were observed when other literature websites were interrogated. For instance, a total of 111 papers was found in the *ISI Web of Knowledge* for the "oxidative stress" and "heavy metals" combination (Table 1) and 91 papers were found for the same keywords using *SCOPUS* for the same period. Although the numbers are not exactly the same, it is obvious that the publication volume increased after the year 2000.

Although it is good to see that researchers around the globe have concentrated their efforts on improving our understanding of the relationship between heavy metals and oxidative stress, it is possible that a significant part of the literature is beginning to show signs of repetition. We could perhaps compare such a situation to what has happened in salt and drought stress studies, which, for a while, were consistently based on the analysis of proline content and variations thereof. It does not mean that measuring proline was not adequate or important, but for some time, papers tended to focus only, or mainly, on this aspect.

Table 1. Number of publications obtained by the use of specific keywords in the ISI Web of Knowledge from 1990 till 2005.

Key words[a]	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
Heavy metals+Plants+Stress	1	5	6	5	5	14	14	18	27	24	22	45	38	46	56	66
Heavy metals+Catalase+Plants	0	0	0	1	0	3	1	1	10	5	9	8	12	17	16	21
Heavy metals +Glutathione+Plants	0	5	5	5	5	7	8	4	14	14	14	17	18	19	22	28
Phytochelatin	6	19	24	18	28	42	31	28	43	40	36	40	55	55	68	64
Superoxide dismutase+Plants	4	34	39	48	61	74	59	92	110	112	105	125	135	167	194	185
Heavy metals+ Plants	14	82	77	97	125	150	145	172	190	215	190	251	244	293	345	408
Heavy metals+Oxidative stress	0	0	0	1	0	0	2	0	6	7	7	15	13	13	20	27
Cadmim+Glutathione+Plants	0	8	15	10	12	24	13	10	18	22	17	25	38	34	36	59

[a] In the "General Search" page, "Keywords" was selected for the search. For example: "Heavy metals AND Plants AND Stress".

During the past five years, we have observed a large number of papers in which exactly the same aspects were analyzed; for example, hundreds of papers reported data on the variations in the activities of antioxidant enzymes. What was varied was the plant species, tissue, stage of development or metal used to induce the stress, but the approach was essentially the same. Again, we do not mean to undermine the merit of measuring the activity of antioxidant enzymes and their importance relative to oxidative stress. On the contrary, such studies have provided extremely important information on the effects of several heavy metals and other abiotic and biotic stresses on plant metabolism.

There is still plenty of room for such analyses; however, we feel that along with such research, other approaches should now be included to consolidate the work on oxidative stress induced by metals, or even other stresses. For instance, very little has been published on research related to proteomics. Even worse is the case for research related to metabolomics to investigate heavy-metal effects in plant metabolism, metallomic analysis of metal binding and metalloproteins (Garcia et al., 2006). Molecular techniques, such as Northern blots and PCR, which have been used to detect the level of gene expression, have been available for quite some time; however, they still do not predominate in this area; their importance in elucidating biochemical problems is undeniable. The new "omics" procedures such as proteomics and metabolomics have expanded very rapidly, despite the complexity of the techniques and, in particular, analysis of the data obtained. An international conference, held in October 2006, on proteomics (bridging the gap between gene expression and biological function), held in Luxembourg (http://proteomlux2006.lippmann.lu/), is a good example of integrative research. Interactomics appears to be the way to validate research findings with different techniques and approaches. For instance, the alteration in the expression of enzyme-proteins in response to a specific stress can be compared with the results of enzyme activity or even gene expression. Such approaches must be adopted rapidly by researchers working on heavy-metal effects on plants.

Mechanisms of detoxification are still a key aspect and must receive more attention; this area of research can benefit from integration of biochemical genetics and plant breeding (mutation or genetic manipulation) to produce stress-tolerant plants. It may also be important to consider a more ecological or ecophysiological and geographical approach that will not only increase our understanding of what is happening in the plant, but also what is happening to a particular geographic area, e.g., urban, arable farm land, forests, rivers and lakes and how they interact with an environmental accidental event. To achieve major advances, geoprocessing and remote-sensing analyses, supported by statistical analyses, may be used to provide a more comprehensive view of the environmental effect caused by pollutants.

One thing that is clear is the need to integrate efforts to use a dynamic and integrative approach, whatever the area of research. This may allow us to complete a chain of interactive and correlated sciences, from interactomics to whole ecosystems. This will improve our understanding of events leading to the current massive environmental contamination, of the effect of the pollutants on plants, animals and humans, and of how to deal with such situations, scientifically and whenever possible, politically. It would be interesting to see in the next few years how research on heavy metals and oxidative stress evolved and which approaches were adopted and found to be most useful.

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