Bull Environ Contam Toxicol (2007) 79:109–113
DOI 10.1007/s00128-007-9143-5

Phytotoxicity of Washing Wastewaters from a Cutlery Production Line

K. Svetková · A. Fargašová

Received: 8 January 2007 / Accepted: 16 March 2007 / Published online: 3 May 2007

Phytotoxicity assessment plays an important role in environmental monitoring and risk assessment of metal-contaminated places. Since, only a few guidelines are available for the assessment of heavy metal phytotoxicity (Römbke and Moltman, 1996), quality-controlled toxicity data using standardized methods are actually quite rarely reported in the literature. Efroymson et al., (1997) developed toxicological benchmarks for screening the effects of contaminants that have the potential to arouse concern. This included the effect of certain heavy metals on terrestrial plants. They also reviewed phytotoxicity data derived from experiments conducted in nutrient culture and spiked soils (Efroymson et al., 1997). Phytotoxicity tests generally use toxicological endpoints such as root and shoot growth, biomass production and germination percent. However, physiological responses of plants to toxic metals consist not only of growth and production inhibition, but also in intensity alterations of various physiological parameters, e.g.. photosynthetic activity, chlorophyll fluorescence and some enzyme activity in plant tissues (Vassilev et al., 1998), which are not yet standardized (Hartley-Whitaker et al., 2001). Also, relationships between metal toxicity and metal tissue concentration have so far been poorly characterized.

K. Svetková
Department of Biotechnology, Faculty of Natural Sciences, University of St. Cyril and Methodius, nám. J. Herdu 2, SK-917 01 Trnava, Slovak Republic

A. Fargašová (✉)
Department of Ecosozology and Physiotactics, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina SK-842 15 Bratislava, Slovak Republic
e-mail: fargasova@fns.uniba.sk

This article describes the chronic toxicity of wastewaters from washing reservoirs of a cutlery production line to terrestrial mustard plant seedlings (*Sinapis alba* L.). Root and shoot growth inhibition, fresh and dry mass production, and water content were used as observed parameters. All contamination of tested washing wastewaters came from heavy metals (Ni, Cr), non-polar extractable compounds (NEC; residues of oils and waxes from polishing of stainless steel cutlery) and detergents (used for cutlery degreasing). Since the tested wastewater was previously classified without verification as dangerous, it is necessary to question the justice of such classification under the new legislation for waste management (Waste Law No. 223/2001, Slovak Republic).

Material and Methods

Mustard (*Sinapis alba* L.) seeds, obtained from Central Control and Testing of the Institute of Agriculture in Bratislava, Slovak Republic, were germinated in Petri dishes with a 9-cm diameter and lined with filter paper. Washing wastewaters for inhibitory tests were used in ten varying concentrations (from 1 to 150 mL/L) and tap water (80 mg/L Ca, 27 mg/L Mg; pH 7.3 ± 0.05) was used for their dilution. In each Petri dish 20 healthy-looking seeds of similar size were spread on filter paper and flushed with 5 mL of tested washing wastewater. Normal tap water was used as the control. The covered Petri dishes were placed in a dark thermostat (25 ^{o}C; air humidity 80%) and, after 72 hr, root and shoot lengths were measured.

Basically the same procedure utilized for growth inhibition was used to determine the dry mass and water content. In these tests, Petri dishes with a 17-cm diameter, and lined with filter paper and a plastic net were used. They contained 50 seeds and 50 mL tested washing water, and they were placed in a dark thermostat. After 72 hr, Petri dishes with germinated seeds were transferred from the thermostat into the laboratory box with a day-light cycle and a constant temperature of 23 ± 1°C. The dishes were shielded from direct sunlight and cultivation lasted for the next 7 days. After 10 days growth (3 + 7) the plants were divided into roots and shoots and the fresh mass was immediately weighed. The plant material was then dried in a drying plant (80°C) to a constant weight. The water content of the plants was determined on the basis of fresh and dry mass using Drazic and Mihailovic's equation (2005):

$$WC = (FM - DM)/DM$$

where *WC*, water content; *FM*, fresh mass; *DM*, dry mass; in g/g *DM*).

The tested samples comprised three different samples of wastewater from washing reservoirs of a cutlery production line mainly polluted by heavy metals (Cr, Ni). Heavy metal content in wastewater is required to be disposed of as hazardous liquid waste. The total metal and non-extractable organic compounds (NEC) contents are shown in Table 1. The first two reservoirs (R1, R2) collected wastewaters from degreasing baths, where the cutlery was degreased from residual oils and furniture creams (Polo Titan produced by Triumph Partizánske, Slovakia – mixture of tensides, alkali and water), while the third reservoir (R3) collected waters from the final cutlery washing pool.

All toxicity tests were carried out in triplicate, and they included a tap water control. Chronic toxicity was assessed by the inhibition of root and shoot growth, and the results were evaluated by the Gryck-Haustein method. IC_{50} concentrations and their 95% confidence intervals (CI) were determined. The results were statistically evaluated using the Toxicity 3.01 and the Adstat 2.0 programs. A *t*test was used to assess the significant difference between the control and other treatments ($P \leq 0.05$). The data was expressed as the average ± the standard deviation (SD).

Table 1 Composition of tested washing wastewater from cutlery production line

Sample	Cr (mg/L)	Ni (mg/L)	NEC (mg/L)
R1	41.6	50.2	1.78
R2	18.8	6.52	2.24
R3	0.3	0.255	6.49

NEC, non-polar extractable compounds

Results and Discussion

This study was carried out to determine the adverse effects of wastewater from washing reservoirs of a cutlery production line to *S. alba* seedlings. Since the main contaminants in these waters were Cr and Ni, the adverse effects were related to these heavy metals. The deleterious effect was expressed as root and shoot growth inhibition couched in regression analysis calculated IC_{50} values (Table 2). On the basis of these values, and their statistical evaluation, waste washingwaters can be arranged for both measured parameters in the following order of inhibition: R1 > R2 >> R3. Both root and shoot prolongation was most inhibited by wastewater from the R1 reservoir, which collected wastewater from degreasing baths. Here, the cutlery was firstly degreased of residual oils and furniture creams. In this reservoir, the metal concentration of Cr and Ni was more than twice as much as that in the R2 reservoir, which collected wastewater from degreasing baths where the cutlery was moved from the R1 reservoir and washed a second time. However, the R3 reservoir collected water from the final cutlery washing–pool, where the metal concentration was very low. It was evident that the toxicity of wastewaters decreased with decreasing metal concentration from the start to the end of the cutlery washing line. All wastewaters reduced more root than shoot growth.

The presence of Cr and Ni in the external environment leads to changes in the growth and development pattern of plants, and both these metals are reported to be very toxic for root and shoot growth (Fargašová, 1994; 1998). Ni in the presence of up 0.1 μM $NiCl_2$ inhibited root and shoot elongation of canola and tomato seedlings, and the roots appeared more sensitive then the shoots (Burd et al., 1998). Prasad et al. (2001) reported that the root length in *Salix viminalis* was affected more by Cr than by Cd and Pb; and Fargašová (1994) stated that the Cr adverse effect on *S. alba*

Table 2 IC_{50} values and their 95% confidence intervals (CI) (mL/L) and Cr/Ni content (mg/L) for wastewaters from cutlery washing line after 72 hr application; the mean of three determinations with a standard deviation 6% or less

	R1		R2		R3	
	IC_{50} (95% CI)	*Cr/Ni*	*IC_{50} (95% CI)*	*Cr/Ni*	*IC_{50} (95% CI)*	*Cr/Ni*
Root	8.35 (6.83–9.72)	0.35/0.42	17.66 (17.17–18.3)	0.33/0.12	48.45 (46.76–50.0)	0.15/0.01
Shoot	12.56 (12.1–13.61)	0.52/0.63	14.43 (14.0–15.07)	0.27/0.09	52.46 (50.3–54.48)	0.16/0.01

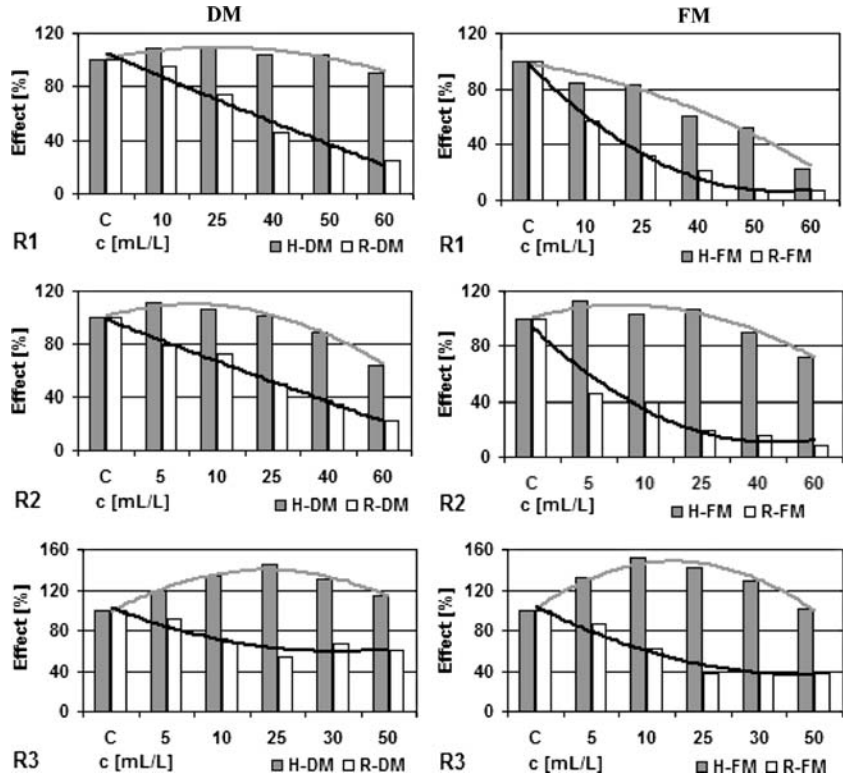

Fig. 1 Dry (DM) and fresh mass (FM) production [%] and their polynomic trend lines after 10 days growth in the presence of tested washing wastewaters (H, shoot; R, root; C, control); mean of three determinations, standard deviation 6% or less

root growth was equal to that of Hg, and stronger than that of Cd and Pb, while Ni reduced root length less than Cr (Fargašová, 1998). The EC_{50} values established by Fargašová (1998) were at least 10 times higher than those observed for washing wastewaters from this cutlery production line. These values indicate a strong synergistic effect between Cr and Ni, as well as a NEC supported adverse effect. However, root growth was reported as a more sensitive indicator of metal toxicity than shoot growth (Burd et al., 1998; Chatterjee and Chaterjee, 2000). Herein, this was significantly confirmed only for washing water from the R1 reservoir. The general response of decreased root growth due to Cr and other metal toxicity may be evoked by the inhibition of root cell division/root elongation or by the extension of the cell cycle in the roots. Under high concentrations of Cr, Ni and many other heavy metals, the reduction of root growth may be due to the direct contact of seedlings roots with metal present in the medium, causing collapse, and subsequent inability of roots to absorb water from the medium (Barcelo et al., 1986).

Adverse effects of Cr, Ni and other metals on plant height and shoot growth were published by Rout et al. (2000), Barton et al. (2000) and Sharma and Sharma (1996). The significant reduction in plant height of *S. alba* observed in this present research was also reported for this plant by Hanus and Tomas (1993) when the Cr level was present at concentrations of 200 or 400 mg/kg soil, with accompanying N, P, K and S fertilizers. The reduction in plant height may be due mainly to the reduced root growth and consequent lesser nutrient and water transport to the shoots. Additionally, Cr and Ni transport to the aerial part of plant can have a direct impact on the cellular metabolism of shoots, thus contributing to the reduction in plant height (Shanker et al., 2005).

The main prerequisite for a higher yield in plants is an increase in biomass production in terms of dry matter. Tested washing wastewaters influenced the dry and fresh mass production of both parts of *S. alba* seedlings (Fig. 1). With increasing wastewater concentration, a reduction in root dry mass was observed for all washing waters, whereas the dry mass of shoots in the majority of applied concentrations was either not affected, or it increased. The washing water from reservoir R3 showed the strongest stimulatory effect mainly on shoot growth – 140% more than that on the control, even in a concentration of 25 mL/L. A reduction in shoot dry mass was determined only for wastewaters from the R1 and R2 reservoirs in concentrations of higher than 60 and 40 mL/L, respectively (Fig. 1, DM). The effect of tested washing waters was stronger on fresh mass production than on dry mass production (Fig. L, FM). This indicates problems in water reception and translocation. Again, root fresh mass production was reduced more strongly than that of shoots. Only washing water from the R1 reservoir seriously reduced the production of shoot fresh mass. The weakest inhibitory effect was observed in washing water from the

R3 reservoir. These results agree with those of the cabbage (*Brassica oleacera* L.) reported by Pandey and Sharma (2002), where the root dry mass was diminished by a nickel excess. They also correlate with those stated by Bennicelli et al. (2004) for the water fern *Azolla caroliniana*, with its biomass reduction in the presence of chromium. It was found that dry matter production in *Vallisneria spiralis* L. was severely affected by Cr(VI) concentrations above 2.5 μg/mL in the nutrient medium (Vajpayee et al., 2001), which indicates at least a 10 times higher value than that for the adverse effects of washing waters on *S. alba* dry mass production. Zurayk et al. (2001) reported that salinity and Cr(VI) interaction caused a significant decrease in the dry biomass of *Portulaca oleracea*; and Chatterjee and Chatterjee (2000) found a restriction in dry biomass production in the cauliflower (cv. Maghi) when it was cultivated in 26 mg/L Cr(VI). Kočík and Ilavský (1994) stated that dry matter production in sunflower, maize and *Vicia faba* was not markedly affected in 200 mg Cr(VI)/kg soil. Supporting the experiments with young seedling described here, a distinct reduction in dry biomass was also reported by Hanus and Tomas (1993) for the flowering stage of *S. alba* when Cr(VI) was introduced at rates of 200 or 400 mg/kg soil along with N, P, K and S fertilizers. *Phaseolus vulgaris* and maize plants exposed to 1 μM Cr(III) showed a higher root and leaf dry weight (DW) than controls, and this DW increase was more pronounced in Fe-deficient conditions (Barcelo et al., 1993). In this study of *S. alba*, the increase in dry mass was exhibited in the majority of washing wastewater concentrations utilized.

The effect of Cr and Ni on plant processes during their early growth and development culminates in a reduction of yield and total dry mass due to poor production, translocation and apportioning of assimilates to the economic parts of the plant. The negative effect on yield and dry matter is essentially an indirect effect of Cr and Ni on plants. The overall adverse effect of Cr on the growth and development of plants may be serious impairment of the uptake of mineral nutrients and of water, which leads to deficiency in the shoots. Wilting of various crops and plant species due to Cr toxicity has been reported (Turner and Rust, 1971), but little information is available on the exact

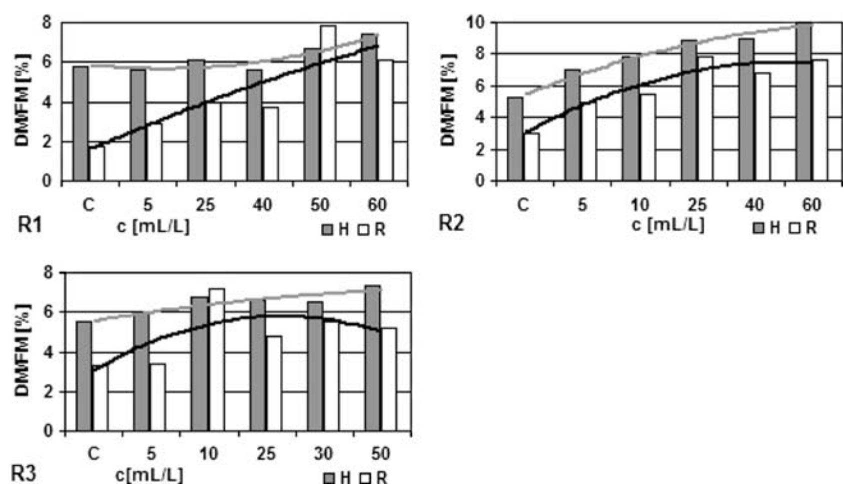

Fig. 2 Relation between dry (DM) and fresh mass (FM) [%] and their polynomic trend lines after 10 days growth of *S. alba* seedlings in the presence of tested washing wastewaters from a cutlery production line (H, shoot; R, root; C, control); mean of three determinations, standard deviation 6% or less

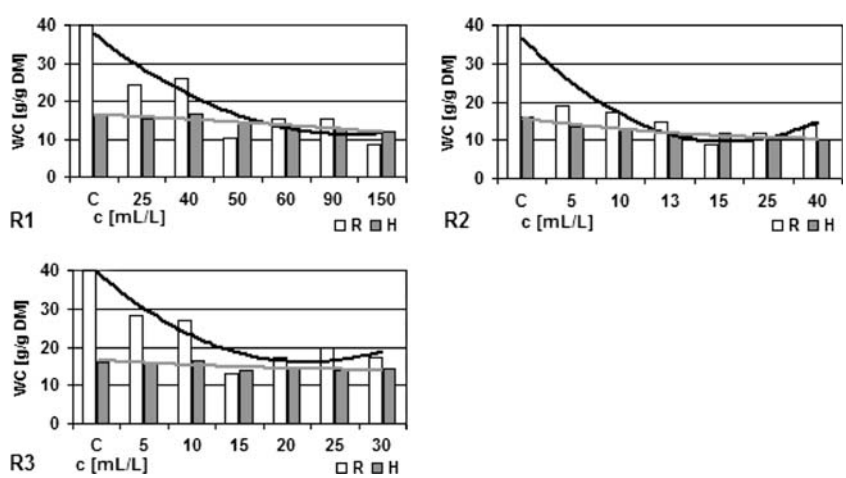

Fig. 3 Water content (g/g DM) in roots and shoots of *S. alba* seedlings and their polynomic trend lines after 10 days growth in the presence of tested washing wastewaters from a cutlery production line (H, shoot; R, root; C, control); mean of three determinations, standard deviation 6% or less

effect of Cr and Ni on water relationships in higher plants. When the relationship between dry and fresh mass was determined herein (Fig. 2), the dry mass fraction increased as the concentration of tested washing water increased. This indicates a reduction in water uptake and translocation through the plant. Water content reduced very rapidly in comparison to that in control seedlings, and this occurred mainly in the roots where water content varied with tested water concentrations (Fig. 3). Water content in the shoots was not significantly reduced in the presence of tested wastewaters (Fig. 3). It can be concluded that tested washing wastewaters containing Cr and Ni inhibited water absorption by the root, but not water translocation into the upper seedlings parts. These results agree with the Chatterjee and Chatterjee (2000) conclusion, that excess Cr decreases the water potential and transpiration rates, and increases diffusive resistance and relative water content in cauliflower leaves. However, Barcelo et al. (1985) observed a decrease in leaf water potential in a Cr treated bean plant. Decreased turgor and plasmolysis was also observed in the epidermal and cortical cells of bush bean plants exposed to Cr, because toxic levels of Cr decreased tracheary vessel diameter, thereby reducing longitudinal water movement (Vazques et al., 1987).

Tests confirmed that the toxicity of the tested wastewaters declined in direct relationship to the decrease in Cr and Ni content. Since the toxicity of the presented wastewaters from the metal surface finishing was confirmed, they were designated as hazardous waste by legally assigned persons.

Acknowledgements This study was supported by grants VEGA 1/4361/07.

References

Barcelo J, Poschenrieder C, Ruano A, Gunse B (1985) Leaf water potential in Cr(VI) treated bean plants (*Phaseolus vulgaris* L.). Plant Physiol Suppl 77:163–164

Barcelo J, Poschenrieder C, Gunse B (1986) Water relations of chromium VI treated bush bean plants (*Phaseolus vulgaris* L. cv. Contender) under both normal and water stress conditions. J Exp Bot 37:178–187

Barcelo J, Poschenrieder C, Vazquez MD, Gunse B, Vernet JP (1993) Beneficial and toxic effects of chromium in plants: solution culture, pot and field studies. Studies in Environmental Science. Proc. 5th Int Conf Environmental Contamination, Morges, Switzerland

Barton LL, Johnson GV, O'Nan AG, Wagener BM (2000) Inhibition of ferric chelate reductase in alfafa roots by cobalt, nickel, chromium, and copper. J Plant Nutr 23:1833–1845

Bennicelli R, Stępniewska Z, Banach A, Szajnocha K, Ostrowski J (2004) The ability of *Azolla caroliniana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. Chemosphere 55:141–146

Burd GI, Dixon DG, Glick BR (1998) A plant growth-promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668

Chatterjee J, Chatterjee C (2000) Phytotoxicity of cobalt, chromium and copper in cauliflower. Environ Pollut 109:69–74

Drazic G, Mihailovic N (2005) Modification of cadmium toxicity in soybean seedlings by salicylic acid. Plant Sci 168:511–517

Efroymson RA, Will ME, Suter GW, Wootten AC (1997) Toxicological benchmarks for screening contaminants of potential concern for effects on terrestrial plants. Office of Environmental Management, US Department of Energy

Fargašová A (1994) Effect of Pb, Cd, Hg, As, and Cr on germination and root growth of *Sinapis alba* seeds. Bull Environ Contam Toxicol 52:452–456

Fargašová A (1998) Root growth inhibition, photosynthetic pigments production, and metal accumulation in *Sinapis alba* as the parameters for trace metals effect determination. Bull Environ Contam Toxicol 61:762–769

Hanus J, Tomas J (1993) An investigation of chromium content and its uptake from soil in white mustard. Acta Fytotech 48:39–47

Hartley-Whitaker J, Ainsworth G, Meharg AA (2001) Copper and arsenate induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. Plant Cell Environ 24:713–722

Kočík K, Ilavský J (1994) Effect of Sr and Cr on the quantity and quality of the biomass of field crops. Proc Semin Production and Utilization of Agricultural and Forest Biomass for Energy, Zvolen, Slovakia, pp168–178

Pandey N, Sharma Ch P (2002) Effect of heavy metals Co^{2+}, Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. Plant Sci 163:753–758

Prasad MNV, Greger M, Landberg T (2001) *Acacia nilotica* L. bark removes toxic elements from solution: corroboration from toxicity bioassay using *Salix viminalis* L. in hydroponic system. Int J Phytoremed 3:289–300

Rout GR, Sanghamitra S, Das P (2000) Effects of chromium and nickel on germination and growth in tolerant and non-toletant populations of *Echinochloa colona* (L.). Chemosphere 40:855–859

Römbke J, Moltman JF (1996) Applied ecotoxicology. CRC, Boca Raton, FL

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31:739–753

Sharma DC, Sharma CP (1996) Chromium uptake and toxicity effects on growth and metabolic activity in wheat, *Triticum aestivum* L. cv. UP 2003. Indian J Exp Biol Plant Sci 34:689–691

Turner MA, Rust RH (1971) Effects of chromium on growth and mineral nutrition of soybeans. Soil Sci Soc Am Proc 35:755–758

Vajpayee P, Rai UN, Ali MB, Tripathi RD, Yadav V, Sinha S (2001) Chromium induced physiological changes in *Vallisneria spiralis* L. and its role in phytoremediation of tannery effluent. Bull Environ Contam Toxicol 67:246–256

Vassilev A, Berowa M, Zlatev Z (1998) Influence of Cd^{2+} on growth, chlorophyll content, and water relations in young barley plants. Biol Plant 41:601–606

Vazques MD, Poschenrieder Ch, Barcelo J (1987) Chromium (VI) induced structural changes in bush bean plants. Ann Bot 59:427–438

Zurayk R, Sukkariyah B, Baalbaki R (2001) Common hydrophytes as bioindicators of nickel, chromium and cadmium pollution. Water Air Soil Pollut 127:373–388