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Impact of biosolids and wastewater effluent application to agricultural land on steroidal hormone content in lettuce plants

Dorit Shargil *, Zev Gerstl, Pinchas Fine, Ido Nitsan, Daniel Kurtzman

Institute of Soil, Water and Environmental Sciences, The Volcani Center, Agricultural Research Organization, POB 6, Bet Dagan 50250, Israel

HIGHLIGHTS

- Increased concentrations of steroidal hormones in soils and water are considered a major concern to human health.
- The study analyzed estrone and testosterone in lettuce plants under different irrigation and sludge application conditions.
- Considerable concentrations of the hormones were found in the lettuce plants, wastewater, freshwater and biosolids.
- Irrigation water had the most substantial effect, whereas biosolids had only minor influence on hormone levels.
- The hormone levels in the plants exceeded the FDA recommendation, and therefore could have negative physiological impacts.

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ABSTRACT

One of the major concerns for human health in the past decade is the potential dangers posed by increased concentrations of steroidal hormones in soils and water. These hormones are considered to be endocrine disrupting compounds (EDCs), which may harm human health when exposed to high concentrations, or in the case of long term exposure to lower concentrations. In a 3 year study, two steroids, estrone and testosterone, were measured in lettuce plants irrigated with wastewater effluents and freshwater and treated with several types of biosolids. The relative contribution of the different factors, mainly irrigation water and biosolids, to the hormone levels in the lettuce plants was determined. It was found that irrigation water, which contained significant amounts of hormones, had the most substantial effect, whereas biosolids had only minor influence on hormone levels in the lettuce.

The hormone levels in the plants were compared to the FDA recommendation for daily consumption in food, and were found to exceed the recommended level (when consumed by a typical individual), and therefore could have negative physiological impacts.

Overall this study shows that biosolids have little effect on hormone uptake by lettuce, and it emphasizes the negative impact of irrigation water on these levels, which is of concern to public health.

1. Introduction

One of the major concerns regarding human health in the past decade is the potential dangers posed by increased levels of steroidal hormones in soils and water. These steroids are considered to be endocrine disrupting compounds (EDCs) and have been identified as the compounds responsible for androgenic activities, in wastewater effluents, sewage sludge and runoff from livestock feeding areas (Hoffmann and Evers, 1986; Kolodziej et al., 2003; Orlando et al., 2004).

There are many advantages to the use of sludge products, which contain nutrients and organic matter which may enhance soil properties and plant yield. It also solves the ecologic problem of waste disposal. Therefore, efforts are being made to increase the reuse of sludge products in agricultural fields. For example, about half of the sludge produced in the largest sewage treatment plant in Israel (Shafdan) is used in agriculture as class A biosolids (sludge that has been treated to reduce bacteria prior to application to land; Harrison and Oakes, 2002). Israel is also one of the leading countries in exploitation of effluents for irrigation — in 2010, 86% of the total effluents were reused in agriculture (Israel Water Authority, 2012).

The reuse of sludge products must be undertaken with consideration of the potential risk to environmental and human health

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* Corresponding author. Tel.: +972 54 3190024.
E-mail address: doritsh@volcani.agri.gov.il (D. Shargil).

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posed by microbial, organic and inorganic contaminants that may be found in the material. The importance of controlling the presence of these hormones in sewage sludge, effluents and even in fresh water is that these contaminants can find their way into the food chain.

In recent decades considerable evidence indicates that exposure to estrogen, either from an external source or endogenous, increases the chance of breast cancer (Zumoff, 1998). Exposure to estrogen can also cause early sexual maturation in girls (Aksglaede et al., 2006).

Numerous studies have shown the link between prostate cancer and testosterone levels (e.g. Bassil et al., 2009), and indeed part of the treatment of prostate cancer is hormonal or physiological castration to reduce the negative impact of testosterone.

Uptake of polluting chemicals by plant roots, and their translocation into plant tissue above ground, are critical to assessing potential risks to human health and ecological damage. Most organic pollutants are absorbed passively by the plant through the roots along with water (transpiration). This means that both the concentration of the pollutant in the soil and irrigation water, and the amount of available water are factors in increasing chemical uptake by plants (Dettenmaier et al., 2009).

There are numerous studies on the uptake of PPCPs (pharmaceuticals and personal care products) by plants (Wu et al., 2010; Holling et al., 2012), but little information is available on the uptake of EDCs. The results of acute toxicity tests which have been conducted for some of the steroid hormones show that the concentration level found in crops was negligible for possible physiological effects if consumed (Sabourin et al., 2012). However, to the best of our knowledge, there is no data available in the literature on the long-term exposure of soil to hormones which may ultimately result in amounts of hormones in the plants to levels which could cause disruption of reproductive or metabolic functions.

Many data gaps still remain regarding the uptake of steroids by plants. We therefore determined if measurable amounts of the two steroid hormones (estrone and testosterone) were present in the lettuce plants, and if long term exposure to biosolids or wastewater effluents could increase their concentration. This was done using protocols for irrigation with fresh water or wastewater effluents in soils amended with three types of biosolids at two sites for three years. The two steroids were selected as a preliminary survey indicated that they were measurable in lettuce, and the enzyme-linked immune-sorbent assay (ELISA) could reliably measure them. Lettuce was selected as it absorbs about half its weight in water and can be grown in both summer and winter.

2. Materials and methods

2.1. Experimental setup

Estrogen and testosterone were monitored in lettuce plants that were grown in lysimeters. The treatments included three different soils, thus ensuring no prior influence on the test system. The treatments constituted amending the different soils with three types of biosolids, and irrigation with either tap water or wastewater effluents. The biosolids were applied three times at a loading rate in accord with their nitrogen content, usually at a 500 kg total N ha^{-1} loading. The biosolids were applied in three consecutive summers, and lettuce plants were planted two weeks after the application (two summer cycles) and in the following December (two winter cycles). Following the first application (in May 2011), corn was sown (not reported here). The wastewater effluent-irrigated lysimeters were located near a wastewater effluent irrigated field site (Kibbutz Revadim) and the tap water irrigated lysimeters were located in the ARO-Volcani campus at Bet Dagan, Israel.

2.2. Lysimeter construction

The lysimeters were constructed from cylindrical plastic high-density polyethylene (HDPE) barrels (Pachmas, Ein Hahoresh, Israel), of the following dimensions: 220 liter volume, 0.264 m^2 surface area, 90 cm depth and 10 kg weight. The barrels were painted white to reduce heating. Each barrel was equipped with a drainage device made of a 2 inch diameter pipe screwed to the side of the container near its bottom. The length of the pipes was 80 cm (lysimeters packed with the clayey soil) or 50 cm (lysimeters packed with sand and loessial soil). The inside of the lysimeters' bottoms were reshaped by covering them with concrete forming a 5–10° inclination toward the drain pipe. The pipes were filled with rock wool, which also extended (a 2 kg portion) into the containers forming a thin layer on their bottom. The inclination and the rock wool were introduced in order to improve drainage (Ben-Gal and Shani, 2002). The lysimeters were placed in groups of four on metal benches (3 m long, 60 cm wide and 60 cm tall) (Fine et al., 2002).

Irrigation was computer controlled. In the summers, irrigation at the Revadim site was with secondary effluents and at the ARO-Volcani tap water was used. In the winter, when rain was inadequate, supplemental irrigation was with tap water at both sites. The soil matrix-tension at Revadim was monitored with digital transmitting (using cellular communication) tensiometers (Mottes, Israel) placed at 30-cm depth in 3 lysimeters — one for each of the three soils. At the ARO-Volcani site, three lysimeters were placed on top of 600-kg electronic scales (Shekel, Rosh Ha'Ain, Israel) connected to a multi-purpose data logger (CR1000, Campbell Scientific).

2.3. Soils

Three soils were used: a clayey soil from Revadim (thermo Chromic Haploxerolls; sand 370 g kg^{-1}, silt 210 g kg^{-1}, and clay 420 g kg^{-1}), a loessial sandy clay loam from Nahal Oz (mixed thermic Calcic Haploxeralf; sand 510 g kg^{-1}, silt 200 g kg^{-1}, and clay 290 g kg^{-1}), and the third soil matrix was a quartzite dune sand (sand 940 g kg^{-1}, silt 10 g kg^{-1}, and clay 50 g kg^{-1}) from the Mediterranean Coastal Plains, Israel. The soils were collected from the 0–20 cm layer. After the addition of the biosolids the soils were analyzed for availability of mineral N, P and K, and fertilizers were applied according to local recommendations.

2.4. Biosolids

Three biosolids were used (Table 2): class A composted sludge (Shacham, Delila, Israel), a class A N-Viro Soil product (NVS) made from municipal sludge reacted with lime and coal-burning fly ash at a 50:4:46 (w/w) ratio, and anaerobically digested, excess activated class B sludge (Haifa, Israel). Once a year (in May — 2011, 2012, 2013), biosolids were mixed into the upper 15-cm layer of the soils by removing this soil layer, mixing it well with the biosolids and re-packing it into the lysimeter. The biosolids were applied at a rate equivalent to application of 500 kg N ha^{-1}. NVS was also applied at 1500 kg N ha^{-1}. The biosolid amounts actually mixed into the soil were calculated based on the concentration of total nitrogen in the product and according to the surface area of the lysimeter (0.264 m^2). Hence, the average amounts applied per year at the 500 kg N ha^{-1} were: 590, 580, and 770 g/lysimeter of compost, 1700, 1300, and 1500 g/lysimeter of NVS and 237, 211, and 1160 g/lysimeter of class B biosolids, and 5.1, 3.96 and 4.5 kg NVS/lysimeter at the 1500 kg N ha^{-1} treatment.

2.5. Treatments

Two sets of lysimeters were operated (Table A.1): a set of 40 filled with the three types of soil at the Kibbutz Revadim site, and a set